SPONDYLOARTHRITIS

IL-23 inhibitor guselkumab shows promise for PsA

> The primary end point … was met for both guselkumab dosing regimens in both trials

The results of the DISCOVER-1 and DISCOVER-2 trials — two phase III trials assessing the IL-23 inhibitor guselkumab as a treatment for psoriatic arthritis (PsA) — suggest that guselkumab is efficacious in the treatment of PsA and could provide an additional treatment option for patients with this disease.

"Evidence from preclinical models suggested that the IL-23–T helper 17 (T_H17) pathway has a predominant role in the pathogenesis of inflammatory disease including psoriasis and PsA," explains Atul Deodhar, corresponding author on the DISCOVER-1 trial. "These latest results confirm the potential benefit of targeting the IL-23 cytokine in the treatment of PsA," continues corresponding author on the DISCOVER-2 trial, Philip Mease.

Guselkumab selectively targets IL-23 by binding to its p19 subunit. This drug is already approved for the treatment of patients with moderate-to-severe psoriasis and, in a previous phase II proof-of-concept study, improved signs and symptoms of PsA, leading to these latest two trials.

The DISCOVER-1 trial aimed to assess guselkumab in patients with a broad range of baseline levels of disease activity. The enrolled patients had active PsA (at least three swollen and three tender joints and CRP ≥0.3 mg/dL) and included patients who had an inadequate response or intolerance of standard treatment (including apremilast, DMARDs or NSAIDs) or TNF inhibition.

"Allocating approximately 30% of the study participants to individuals previously treated with one or two TNF inhibitors enabled the evaluation of guselkumab in patients who require a different mechanism of action to safely and effectively treat all aspects of their disease," reports Deodhar.

By contrast, the larger DISCOVER-2 trial aimed to assess guselkumab in biologic-naive patients with active PsA (at least five swollen joints, at least five tender joints and CRP ≥0.6 mg/dL), despite standard therapies.

"In both trials, two guselkumab dosing regimens were studied: the regimen that is approved to treat psoriasis and that showed efficacy in the phase II proof-of-concept study (guselkumab 100 mg given every 8 weeks), as well as a more frequent dosing interval (guselkumab 100 mg given every 4 weeks) to evaluate whether higher serum concentrations would afford greater efficacy in PsA," explains Deodhar.

The primary end point (the proportion of patients who achieved an ACR20 response (≥20% improvement in ACR criteria) at week 24) was met for both guselkumab dosing regimens in both trials. In the DISCOVER-1 trial, 59% of patients in the every 4 weeks group and 52% of patients in the every 8 weeks group had an ACR20 response at week 24 compared with only 22% of patients in the placebo group. Similarly, 64% of patients in both the every 4 weeks group and the every 8 weeks group had an ACR20 response in the DISCOVER-2 trial, compared with 33% of patients in the placebo group. The percentage differences versus placebo were statistically significant for all the treatment groups across both studies ($P < 0.0001$).

"The most notable findings were that guselkumab was shown to benefit multiple clinical domains of PsA in both of these study populations," says Mease. Treatment with guselkumab (using either regimen) improved joint and skin symptoms as well as physical function and health-related quality of life in both trials. Furthermore, in the DISCOVER-2 trial, the 4-week regimen could inhibit progression of structural damage versus placebo, as assessed by changes in the PsA-modified van der Heijde-Sharp (vdHS) score at week 24.

Analysis of pooled data from DISCOVER-1 and DISCOVER-2 also revealed that a higher proportion of patients in either treatment group had clinically resolved dactylitis and enthesitis at week 24 than in the placebo group.

Both guselkumab regimens had a favourable safety profile that was consistent with the safety profile observed in the treatment of patients with psoriasis. These trials are being extended (DISCOVER-1 for 1 year and DISCOVER-2 for 2 years) to provide additional data on the efficacy and safety of guselkumab. "Further assessments of combined data from both trials are also planned, including integrating guselkumab safety data across trials and assessing the effects of guselkumab on PsA axial disease," says Deodhar.

"An important question to be addressed is whether IL-23 inhibition can benefit axial inflammation in patients with PsA, a clinical domain not typically addressed in PsA trials," states Mease. "Data on patients with PsA spondylitis were collected in the DISCOVER programme and will be presented at upcoming meetings."

Jessica McHugh

ORIGINAL ARTICLES Deodhar, A. et al. Guselkumab in patients with active psoriatic arthritis who were biologic-naive or had previously received TNFα inhibitor treatment (DISCOVER-1): a double-blind, randomised, placebo-controlled phase 3 trial. *Lancet* **395**, 1115–1125 (2020) | Mease, P. J. et al. Guselkumab in biologic-naive patients with active psoriatic arthritis (DISCOVER-2): a double-blind, randomised, placebo-controlled phase 3 trial. *Lancet* **395**, 1126–1136 (2020)

SPONDYLOARTHRITIS

TYK2 inhibition halts SpA

Targeting the Janus kinase (JAK) TYK2 could have disease-modifying effects in spondyloarthritis (SpA) by halting inflammation and bone erosion, according to new research. In the study, a novel small-molecule inhibitor of TYK2 blocked IL-23 signalling in vitro and inhibited disease progression in mouse models of SpA.

The researchers first showed that the selective TYK2 inhibitor NDI-031407 inhibited IL-23-induced IL-17A production as well as IL-23-induced STAT3 phosphorylation in a dose-dependent manner in human $CD4^+$ T cells. In vivo, in the β-1,3-glucan (curdlan)-triggered SKG mouse model of SpA, treatment with orally delivered NDI-031407 prevented disease progression, as reflected by lower clinical scores for SpA symptoms. Bone erosion, joint space narrowing and bone marrow oedema were also prevented in NDI-031407-treated as compared with vehicle-treated mice. The SpA-associated expansion and activation of T_H17 cells in the draining lymph nodes and arthritic joints of mice was reduced in NDI-031407-treated mice. In a different model of SpA, induced by delivery of minicircle DNA expressing *IL23*, the TYK2 inhibitor also protected against clinical disease driven by systemic IL-23 expression.

Addressing the biologic relevance of *TYK2* single-nucleotide polymorphisms (SNPs) that have been associated with ankylosing spondylitis (AS) in genome-wide association studies, the researchers found that carriage of certain *TYK2* SNPs was associated with a decreased T_H1 cell frequency and a progressive AS phenotype characterized by a high rate of vertebral fusion.

Previous studies have suggested that pan-JAK inhibitors, such as tofacitinib, could be useful for the treatment of SpA. These latest findings suggest that TYK2 has a role in the pathogenesis of AS, and that specific inhibition of TYK2 inhibits disease progression in animal models of SpA. The researchers propose that a small-molecule TYK2 inhibitor could be explored as a potential disease-modifying therapy for AS.

Sarah Onuora

ORIGINAL ARTICLE Gracey, E. et al. TYK2 inhibition reduces type 3 immunity and modifies disease progression in murine spondyloarthritis. *J. Clin. Invest.* https://doi.org/10.1172/JCI126567 (2020)
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GOUT

β-carotene blocks the inflammasome

The NLRP3 inflammasome has a prominent role in the pathogenesis of inflammatory arthritis, particularly gout. Several approaches are currently being explored to target the NLRP3 inflammasome as a strategy for the treatment of gout, most of which target the NACHT domain of NLRP3. A new study that aimed to investigate molecules that target the pyrin domain of NLRP3 suggests that β-carotene might have potential as a future therapy for gout.

"Although the antioxidant activity of β-carotene, a plant-derived provitamin A, has been widely reported, our study is the first report to provide the mechanistic detail as to how β-carotene supplementation might prevent NLRP3 inflammasome-related diseases such as gouty arthritis," states corresponding author Joo Y. Lee.

The researchers began by establishing β-carotene as a promising binding candidate for the pyrin domain of NLRP3. "We ran molecular docking modelling screening with ~62,800 compounds and selected β-carotene as an NLRP3 inflammasome inhibitor, then identified the direct binding mode between β-carotene and the pyrin domain of NLRP3 using surface plasmon resonance analysis and NLRP3 mutation experiments," says Lee.

In mouse models of gouty arthritis of the knee and foot, administration of oral β-carotene prior to injection with monosodium urate crystals reduced the severity of disease and prevented the production of IL-1β and caspase 1 (products of the NLRP3 inflammasome). Furthermore, the administration of β-carotene to cells from the synovial fluid of patients with gout reduced their secretion of IL-1β in a dose-dependent manner.

"Our findings suggest that pharmacological application of β-carotene might improve inflammatory symptoms related to the NLRP3 inflammasome, such as those experienced by patients with gout," concludes Lee.

Joanna Clarke

ORIGINAL ARTICLE Yang, G. et al. Direct binding to NLRP3 pyrin domain is a novel strategy to prevent NLRP3-driven inflammation and gouty arthritis. *Arthritis Rheumatol.* https://doi.org/10.1002/ART.41245 (2020)
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RHEUMATOID ARTHRITIS

A drug delivery system with sting

Scientists have developed a new system for delivering drugs to cartilage in which the drug, in this case a glucocorticoid, is linked to a peptide found in the venom of scorpions. In a rat model of arthritis, this drug conjugate could reverse signs of arthritis without evidence of glucocorticoid-related systemic toxicity.

Cystine-dense peptides (CDPs) are a family a miniproteins found in a wide variety of species and are often used for predation or protection. In a biodistribution screen of 42 CDPs from 20 species, the researchers identified a group of CDPs that accumulate and persist for several days in the cartilage of rats and mice. They hypothesized that these molecules could be used to deliver drugs for the treatment of arthritis.

Further experiments found that they could conjugate one of the cartilage-accumulating CDPs, CDP-11R, to a fluorophore or dexamethasone, without substantially altering its localization to cartilage. The researchers made further amendments to the drug conjugate by introducing a labile linker that hydrolyses at a desirable rate in the plasma, on the expectation that steric hindrance could otherwise prevent the therapeutic effects of the conjugated drug.

"Unfortunately, the small amounts of dexamethasone that made it into the bloodstream were sufficient to cause adverse effects that we considered unacceptable for long-term treatment," explains James Olson, corresponding author of the new study. "We switched to triamcinolone acetonide (TAA) as the payload because TAA is rapidly metabolized to an inactive metabolite in the bloodstream." In rats with collagen-induced arthritis, various doses of the CDP-11R–TAA conjugate could reduce inflammation in the arthritic joints, without signs of systemic adverse effects (such as atrophy of the spleen or thymus).

"Our vision is to create a version of this drug candidate that patients would administer to themselves infrequently," explains Olson. "We hope that such a drug could provide arthritis relief with no or few adverse effects."

Jessica McHugh

ORIGINAL ARTICLE Cook Sangar, M. L. et al. A potent peptide-steroid conjugate accumulates in cartilage and reverses arthritis without evidence of systemic corticosteroid exposure. *Sci. Transl Med.* **12**, eaay1041 (2020)

BONE

JAK inhibitors boost bone formation

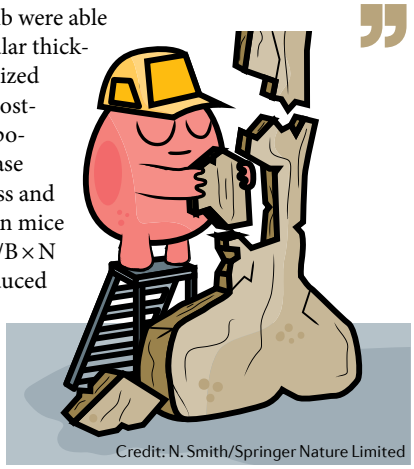
Inhibitors of Janus kinases (JAKs), such as tofacitinib and baricitinib, are used to treat cytokine-mediated inflammatory diseases such as rheumatoid arthritis (RA). Some evidence has emerged to suggest a role for JAK signalling pathways in bone biology, but whether JAK inhibition affects bone remodelling has been uncertain. The results of a new study published in *Science Translational Medicine* shed light on exactly how JAK inhibitors affect bone in health and disease.

"We studied two patients with RA who received 5 mg tofacitinib twice daily," states first author Susanne Adam. "High-resolution peripheral quantitative CT of their metacarpophalangeal joints revealed that tofacitinib treatment reduced bone erosions after 2 years by induction of bone formation."

"Despite the constant progress in anti-rheumatic therapy options, restoring bone tissue that has been subjected to erosive damage by RA has not yet been achieved," explains corresponding author Silke Frey. "Therefore, these findings were unprecedented and encouraged us to investigate the potential bone-anabolic effect of JAK inhibition."

Interestingly, the authors found that 6 weeks of tofacitinib increased bone mass in healthy mice under homeostatic conditions. Both tofacitinib and baricitinib were able to increase trabecular thickness in ovariectomized mice (a model of postmenopausal osteoporosis) and to increase trabecular thickness and cortical thickness in mice with established K/B × N serum transfer-induced arthritis (a model of RA).

> JAK inhibitors reduce bone loss by promoting new bone formation by osteoblasts



Credit: N. Smith/Springer Nature Limited

Delving deeper, the authors discovered that JAK inhibitors reduce bone loss by promoting new bone formation by osteoblasts, rather than by affecting osteoclasts. In vitro experiments and gene network analysis revealed a pathway in which JAK inhibition promotes the stabilization of β-catenin (an important part of the Wnt signalling pathway) and the expression of osteoanabolic genes such as *OCN*, which leads to an increase in the bone mineralization capacity of osteoblasts.

"The capability of JAK inhibitors to directly affect bone metabolism will expand their well-established role as anti-inflammatory agents," says senior author Axel Hueber. "We expect JAK inhibition to not only ameliorate inflammation-induced bone damage, but also to provide additional value in treating osteoporosis-induced loss of bone density, which is a frequent comorbidity of RA."

Joanna Clarke

ORIGINAL ARTICLE Adam, S. et al. JAK inhibition increases bone mass in steady-state conditions and ameliorates pathological bone loss by stimulating osteoblast function. *Sci. Transl Med.* **12**, eaay4447 (2020)

INFLAMMATION

Pro-senescence therapy reduces joint inflammation

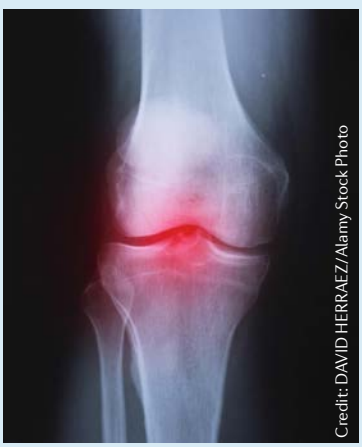
New research shows that inducing senescence of synovial fibroblasts via activation of melanocortin type 1 receptor (MC_1), a G protein-coupled receptor, could offer a novel approach to promote resolution of inflammation in joints affected by rheumatoid arthritis (RA). In the study, administration of a small-molecule MC_1 agonist to synovial fibroblasts from patients with RA not only arrested proliferation of the cells, but also induced a pro-repair phenotype resembling that seen during the remodelling phase of wound healing.

The researchers had previously demonstrated that drugs targeting the pro-resolving melanocortin system had anti-arthritic effects in mouse models of inflammatory arthritis. "In the present work we aimed to translate those findings into human arthritis by studying the effects of melanocortin drugs on human synovial fibroblasts from patients with RA, which are largely responsible for the sustained inflammation and hence lack of resolution within the arthritic joints," explains lead author Trinidad Montero-Melendez.

> BMS-470539 had anti-arthritic effects in association with synovial fibroblast senescence

In the study, treatment of RA synovial fibroblasts with the selective MC_1 agonist BMS-470539, but not with non-selective ligands, induced senescence via phosphorylation of ERK. MC_1 activation modulated processes related to cell cycle regulation, lysosomal function and metabolic processes. BMS-470539-treated cells were also characterized by downregulation of collagens and increased expression of matrix metalloproteinases.

In vivo, in mice with K/B × N serum transfer-induced arthritis, BMS-470539 had anti-arthritic effects in association with synovial fibroblast senescence. Notably, co-administration of senolytic drugs abrogated these anti-arthritic effects.



Credit: DAVID HERRAEZ/Alamy Stock Photo

"Our study shows for the first time that senescence can be induced via the direct activation of a membrane receptor," highlights Montero-Melendez. The results suggest a route to restoring homeostasis to joints affected by RA by directly targeting synovial fibroblasts.

Sarah Onuora

ORIGINAL ARTICLE Montero-Melendez, T. et al. Therapeutic senescence via GPCR activation in synovial fibroblasts facilitates resolution of arthritis. *Nat. Commun.* **11**, 745 (2020)

GOUT

IL-37 linked to gout pathogenesis and treatment

Rare variants of the gene encoding IL-37 have been discovered that result in loss of the cytokine's anti-inflammatory effects and confer predisposition to gout, with additional evidence suggesting that recombinant IL-37 could have therapeutic potential in the disease. "This is a completely new finding in the field of gout and IL-37 biology," notes corresponding author Leo Joosten.

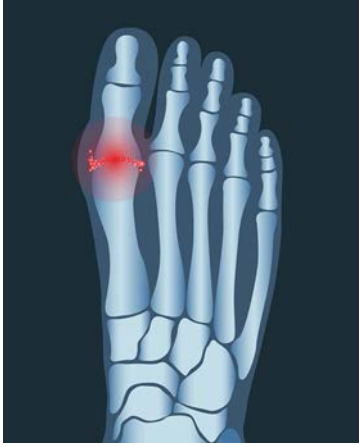
IL-1β-mediated inflammation is central to the pathogenesis of gout. The IL-1 family member IL-37 is known to be a negative regulator of IL-1β signalling, and its anti-inflammatory properties have been demonstrated in numerous disease models. However, the contribution of IL-37 to the disease process in gout was unclear.

In the present study, the researchers identified four distinct rare variants of *IL37* in six patients with gout (within the discovery cohort of 675 patients) using molecular inversion probe-based resequencing technology. The four variants were clustered in exon 5, which encodes the functional domain of IL-37. None of the variants was found in a control cohort of 520 healthy adults.

The researchers also observed that the patients who were carriers of the rare *IL37* variants had either onset of gout at a younger age or a more severe disease phenotype, with multiple inflammatory comorbidities, compared with non-carriers. Predictive modelling and in vitro studies confirmed that the variants affect the structure of the IL-37 protein and consequently its anti-inflammatory function.

In monosodium urate (MSU)-stimulated polymorphonuclear cells from a patient carrying one of the four rare variants, treatment

> "administration of recombinant IL-37 dampened the inflammation induced by MSU crystals"



Credit: maclifethai/iStock/Getty Images Plus

with recombinant IL-37 reduced the production of IL-8 and reactive oxygen species. Moreover, in a mouse model of arthritis, administration of recombinant IL-37 dampened the inflammation induced by MSU crystals in wild-type mice.

"We are planning to perform experiments to treat mice with an IL-37 fusion protein," says Joosten. If those experiments prove successful, the researchers plan to undertake clinical studies to evaluate the treatment in patients with gout.

Sarah Onuora

ORIGINAL ARTICLE Klück, V. et al. Rare genetic variants in interleukin-37 link this antiinflammatory cytokine to the pathogenesis and treatment of gout. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2019-216233 (2020)

SPONDYLOARTHRITIS

Proteinase KLK6 links skin and joint inflammation in PsA

Individuals with psoriasis have an increased risk of developing psoriatic arthritis (PsA), but the exact mechanisms that link skin inflammation and joint disease are unclear. The results of a new study suggest that in mice, signalling between the serine proteinase kallikrein-related peptidase 6 (KLK6) and proteinase-activated receptor 1 (PAR1) in the skin alone might be sufficient to trigger joint disease.

"The idea that overexpressing a serine protease in the epidermis could be sufficient to cause damage to the joints and bones at distant sites was what got us really excited," enthuses corresponding author Nicole Ward. "Preclinical reports have shown that skin-initiated inflammation can lead to arthritis-like changes, so our findings support the idea that chronic inflammation in the skin has the capacity to promote distant damage to the joints and bone, and that eliminating the skin inflammation can reduce that damage."

> "the skin inflammation was necessary for the arthritis to persist"

To investigate the role of KLK6 in psoriasis and PsA, Ward and colleagues developed transgenic mice with overexpression of *Klk6* in their keratinocytes. This overexpression of *Klk6* could be switched off by the administration of doxycycline. These mice, known as *Klk6*$^{+}$ transgenic mice, developed dermatitis that histologically and transcriptionally mirrored human psoriatic skin lesions. Interestingly, the mice also spontaneously developed dactylitis, enthesitis, synovitis, kyphosis and had reduced vertebral bone mineral density, suggestive of a disease similar to that seen in patients with PsA.

Reduction of the *Klk6* expression in these mice to wild-type levels with doxycycline ameliorated the skin inflammation and caused a reversal of at least some of their joint disease, suggesting that the skin inflammation was necessary for the arthritis to persist.

Knockout experiments revealed a role for PAR1, but not for PAR2, in KLK6 signalling, and follow-up experiments on human psoriatic skin explants showed a reduction in the production of pro-inflammatory cytokines upon treatment with the PAR1 inhibitor vorapaxar.

"We are focused on taking this work forward by using the *Klk6*$^{+}$ transgenic mice to study the mechanisms of action by which skin inflammation causes arthritis-like damage and by translating these findings back into mechanisms that are relevant to patients with PsA," concludes Ward.

Joanna Clarke

ORIGINAL ARTICLE Billi, A. C. et al. KLK6 expression in skin induces PAR1-mediated psoriasiform dermatitis and inflammatory joint disease. *J. Clin. Invest.* https://doi.org/10.1172/JCI133159 (2020)
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RHEUMATOID ARTHRITIS

Identifying 'non-progressors' among patients with arthralgia

Axel J Hueber and Gerhard Krönke

Patients with clinically suspect arthralgia have articular symptoms such as pain and stiffness of the small joints without clinical signs of arthritis. Some of these patients progress and develop 'true' disease, but how can we differentiate evolving chronic disease from disease that will resolve?

Refers to ten Brinck, R. M. et al. Improvement of symptoms in clinically suspect arthralgia and resolution of subclinical joint inflammation: a longitudinal study in patients that did not progress to clinical arthritis. *Arthritis Res. Ther.* **22**, 11 (2020).

Clinically suspect arthralgia (CSA) is characterized by inflammatory-type pain of the small joints in the absence of overt clinical inflammation[1], and patients with CSA harbour an increased risk of developing rheumatoid arthritis (RA). However, in clinical practice, predicting which patients with CSA will transition to inflammatory arthritis is difficult. Furthermore, although only a proportion of patients with CSA progress to RA, little is known about the remaining patients with CSA who do not develop this disease. This lack of knowledge is surprising as understanding more about these 'non-progressors' might enable the identification of factors that promote resolution of inflammation or exert protective effects by blocking the onset of RA. A new study by ten Brinck at al.[2] attempts to address this issue by focusing on a cohort of patients with CSA who did not progress to RA over a period of 2 years.

> predicting which patients with CSA will transition to inflammatory arthritis is difficult

ten Brinck at al.[2] performed a series of longitudinal clinical and MRI assessments of patients with newly-onset (<1 year) CSA over 2 years and excluded those patients who progressed to developing RA. At study enrolment, 84% of the patients had signs of subclinical inflammation as determined by MRI, and 19% of the patients were positive for RA-associated antibodies (anti-citrullinated peptide antibodies (ACPAs) or rheumatoid factor). Notably, symptoms resolved in a third of the patients, whereas symptoms persisted, without the development of RA, in the remaining patients at the end of the 2-year period. In accordance with an inflammatory origin of arthralgia in CSA, MRI inflammation scores decreased from baseline in the patients whose symptoms resolved, whereas these scores were unchanged over time in those whose symptoms persisted. Notably, MRI inflammation scores were higher at baseline in patients with CSA whose symptoms subsequently resolved than in those whose symptoms persisted, indicating that increased subclinical inflammation in patients with CSA does not directly correlate with subsequent persistence of symptoms.

Previous research has focussed on identifying the characteristics of those patients with CSA who eventually progress to overt arthritis, which has provided valuable information on the factors and mechanisms that drive onset and progression of inflammatory joint disease (FIG. 1). The factors associated with progression to RA include environmental and genetic risk factors such as smoking and shared epitope positivity, respectively, as well as serologic markers such as ACPAs. Improved MRI techniques have additionally enabled imaging of early phases of disease and have shown that subclinical inflammation, including tenosynovitis, synovitis and osteitis, precede the onset of clinical RA[3]. Furthermore, subclinical bone destruction is already detectable by high resolution CT in ACPA-positive individuals without clinical onset of arthritis[4]. Identification of individuals who are 'at risk' of developing RA is of particular relevance as it might enable preventive therapeutic approaches in these patients. Examples of such preventive approaches include clinical trials of depleting B cells with rituximab[5] or blocking costimulatory molecules with abatacept[6] in preclinical stages of RA.

> both musculoskeletal symptoms and inflammation can spontaneously disappear

In 2005, investigators characterized a distinct synovial fluid cytokine profile that might predict the development of RA in patients with newly-onset arthritis (that is, symptoms of 3 months duration). However, although this profile, characterized by cytokines of stromal and T cell origin, was transient, it was present in patients with disease that had progressed further than CSA, as patients with CSA do not have swollen joints[7]. In the study by ten Brinck et al.[2], the patients with CSA had signs of subclinical inflammation by MRI. Another imaging technique, musculoskeletal ultrasonography, has previously been used to monitor and predict transition from early unclassified arthritis to RA. Musculoskeletal abnormalities detected by ultrasonography were associated with the presence of arthritis in first-degree relatives of patients with RA; thus, these features were detectable in a later stage of disease than CSA[8]. In patients with early unclassified arthritis, musculoskeletal ultrasonography of tenosynovitis has also been used to differentiate patients who will develop persistent RA, patients who will develop non-RA persistent arthritis and patients whose arthritis will resolve[9]. Similar to the MRI data from ten Brinck et al.[2], subclinical inflammation (in this case, ultrasonography-defined tenosynovitis) was present in all the groups. The resolution of arthritis in these patients was associated with negativity for ACPA and rheumatoid factor, and a milder disease at baseline (that is, a lower number of swollen and tender joints), whereas tenosynovitis of

the digit flexors was an independent predictor of the development of persistent RA in this group of patients. Unfortunately, as with most research studies, the main focus of this study was on the patients with disease that progressed rather than patients with disease that resolved.

Although much research has focused on investigating the risk of developing inflammatory arthritis in patients with CSA[1], less attention has been paid to the fraction of patients with CSA who do not develop arthritis. Studies such as the one by ten Brinck et al.[2] can help us understand the mechanisms that prevent the onset of inflammation and contribute to the resolution of arthritis. Indeed, ten Brinck et al.[2] hypothesize that the patients whose symptoms resolved might indeed have had pre-RA, but that one of the several 'final switches' was absent, preventing actual progression to RA.

Overall, the study by ten Brinck et al.[2] provides an important dataset that highlights the connection between arthralgia and subclinical inflammation in patients with CSA who do not progress to RA. Moreover, the findings show that both musculoskeletal symptoms and inflammation can spontaneously disappear. This resolution of symptoms and inflammation in patients with CSA, in turn, did not correlate with the initial degree of subclinical inflammation or seropositivity. This finding complicates approaches for defining the window of opportunity for treatment interventions. Thus, it remains to be addressed which environmental, genetic and immunologic factors contribute to the resolution (or persistence) of symptoms in patients with CSA who do not progress to RA, which is an important area for future research.

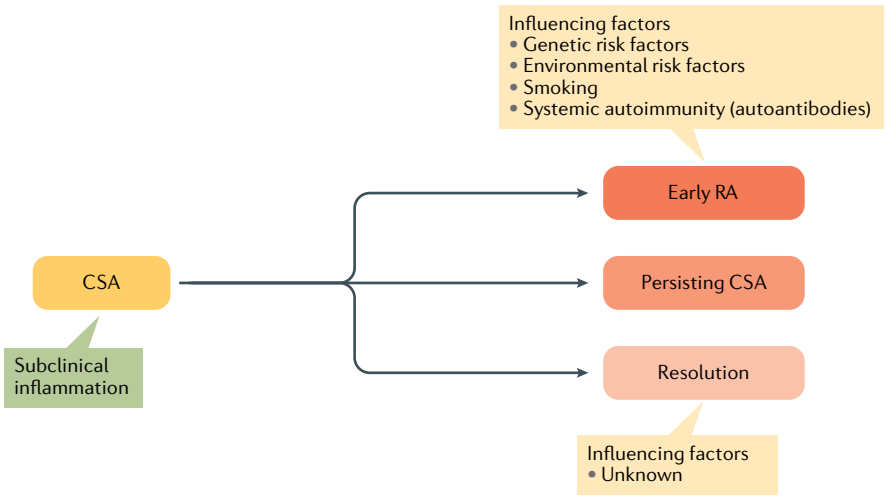

Fig. 1 | **Outcome of clinically suspect arthralgia over time.** Patients with clinically suspect arthralgia (CSA) are at risk of developing rheumatoid arthritis (RA), but a large proportion of these patients do not develop RA. In these latter patients, symptoms of CSA can either persist for years, or can resolve over time. Multiple factors are implicated in the development of RA, whereas factors involved in the resolution of CSA are unknown.

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Competing interests
The authors declare no competing interests.

SYSTEMIC SCLEROSIS

T cells in SSc skin lesions: knowing your enemy

Patrizia Fuschiotti

New research suggests that cytotoxic T cells are dominant in the lesional skin of patients with early diffuse cutaneous systemic sclerosis (SSc) and contribute to vasculopathy and tissue fibrosis. Could therapeutic strategies that prevent T cell activation and cytotoxicity therefore present an option to potentially halt progression of SSc?

Refers to Maehara, T. et al. Cytotoxic CD4+ T lymphocytes may induce endothelial cell apoptosis in systemic sclerosis. *J. Clin. Invest.* https://doi.org/10.1172/JCI131700 (2020).

Systemic sclerosis (SSc) has the highest fatality rate of all connective tissue diseases and is characterized by vascular abnormalities, autoimmunity and fibrosis[1]. Patients with SSc have few therapeutic options — a reflection of our poor understanding of the pathogenesis of SSc. The only therapies currently available to slow disease progression, such as stem cell transplantation and cyclophosphamide, lead to the broad killing of immune cells and consequent toxicities, which can cause death[1]. Therefore, identification of the effector immune pathways that control the pathogenesis of SSc could lead to innovative therapies that selectively target the aberrant immune response, resulting in better efficacy and less toxicity. In a new study, Maehara et al.[2] have quantitatively characterized the immune cell infiltrates in the skin of patients with diffuse cutaneous SSc (dcSSc), which represents a first step towards this goal.

Vascular injury and endothelial damage are the earliest events in the pathogenesis of SSc that are observable by microscopic and immunohistochemical studies of skin samples from patients at various clinical stages[1,3]. This damage is thought to be triggered by viruses, autoantibodies, granzymes or oxidative products[1,3] and is followed by the infiltration of lymphocytes and macrophages into the affected skin, leading to the worsening of vasculopathy and fibrosis. The predominant pro-inflammatory cells in the dermis of patients with SSc are T cells, which are implicated in endothelial cell dysfunction and in the induction of fibrosis[1,3]. Several T cell subsets, including T helper 1 (T_H1) cells, T_H2 cells, T_H17 cells, T_H22 cells, T follicular helper (T_{FH}) cells, regulatory T (T_{reg}) cells and CD8+ T cells, have been implicated in the pathogenesis of SSc[3]; however, a comprehensive and quantitative analysis of SSc lesions has not been previously reported.

In their new study, Maehara et al.[2] used quantitative microscopy-based image analysis to visualize and measure all major CD4+ T cell subsets in the skin of untreated patients with early dcSSc. Their findings indicate that cytotoxic CD4+ T cells (CD4+ CTLs) represent the dominant CD4+ T cell subset infiltrating the skin of most samples analysed, whereas T_H1 cells, T_H2 cells, T_{FH} cells and T_{reg} cells are relatively sparse[2]. Although an increase in T_H17 cells was noted in some samples, the dominant T cell subsets in the majority of samples were cytotoxic T cells, including both CD4+ and CD8+ cells. The discovery of CD4+ CTLs in this context is the major and novel advance of this work[2] and is in agreement with previous studies[4,5] in which accumulated skin-resident effector memory CD8+ CTLs were detected in the skin of patients with early dcSSc.

Maehara et al.[2] show that CD4+ CTLs are characterized by a highly cytotoxic profile (both at protein and transcriptional levels), enhanced metabolic activity and a marked clonal expansion. Importantly, the authors visualized CD4+ CTLs directly in SSc lesions by measuring the co-expression of CD4 and granzyme A. These cells were in the vicinity of endothelial cells that were upregulating HLA-DR molecules and undergoing apoptosis, a well-described phenomenon in the pathogenesis of SSc. Similarly, previous studies in skin from patients with dcSSc have shown that cytotoxic CD8+ T cells co-expressing granzyme B are located in perivascular areas in close proximity to small blood vessels[5], and that CD8+CD226+ T cells are associated with endothelial cell injury[6], thereby supporting the hypothesis that both CD4+ and CD8+ CTLs might induce the apoptosis of endothelial cells in SSc.

In addition to showing endothelial cell apoptosis, Maehara et al.[2] reported a general increase in the apoptosis of non-endothelial cells in dcSSc skin compared with healthy

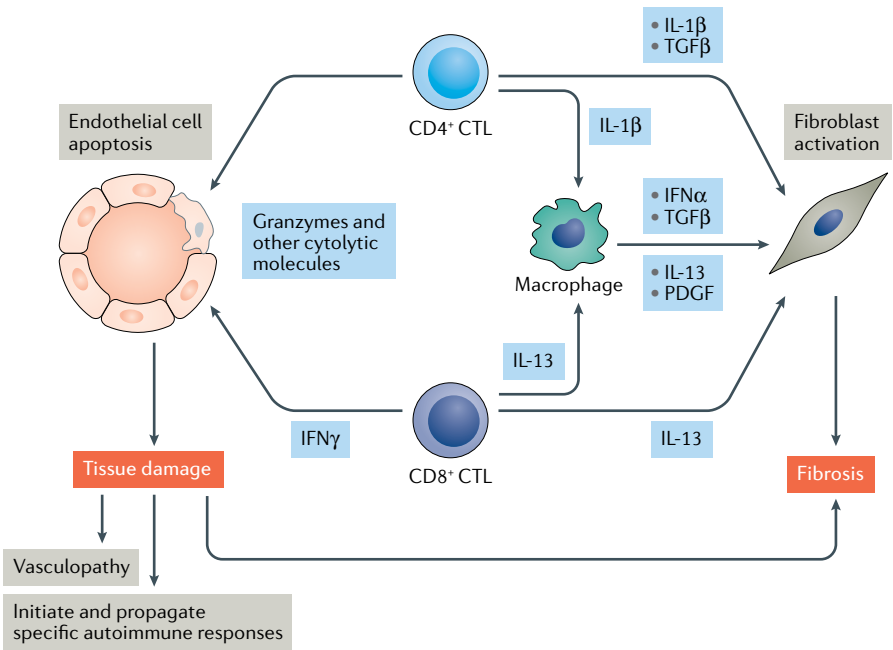

Fig. 1 | **Model for the role of cytotoxic T cells in diffuse cutaneous systemic sclerosis.** Cytotoxic CD4+ T cells (CD4+ CTLs) and CD8+ CTLs in skin lesions in diffuse cutaneous systemic sclerosis might contribute to vasculopathy and tissue fibrosis through targeted endothelial cell killing and by secreting pro-fibrotic cytokines such as IL-1β, transforming growth factor-β (TGFβ) and IL-13. These cytokines induce fibrosis directly by activating fibroblasts or by activating macrophages that in turn produce TGFβ and IL-13, as well as additional pro-fibrotic cytokines (such as IFNα and platelet-derived growth factor (PDGF)).

skin, suggesting that cytotoxic T cells might induce the apoptosis of specific cell types in an antigen-specific manner. Although the antigen-specificity of expanded T cell clones is currently unknown, Maehara et al. suggest that specific self-proteins known to be expressed at high concentrations by endothelial cells (such as CENPA, CENPB and IFI16)[7] might provide peptides that are presented by HLA molecules for T cell recognition, which could contribute to loss of peripheral T cell tolerance and initiate autoimmune responses in draining lymph nodes. Activated $CD4^+$ and $CD8^+$ CTLs might then attack host cells in the tissues of patients with SSc, particularly endothelial cells.

Maehara et al.[2] also showed that $CD4^+$ CTLs from the blood of patients with dcSSc have an activated effector phenotype characterized by loss of expression of the co-stimulatory receptor CD28 (which is usually downregulated upon chronic antigen stimulation) and gain of expression of CD57 (a marker of T cell senescence and cytotoxicity). Similarly, previous studies have shown that activated effector memory $CD8^+CD28^-$ T cells can be found in the blood and skin of patients with early dcSSc, and that these cells express skin-homing receptors[5]. Maehara et al. also investigated the role of the ongoing T cell activation that is a feature of early dcSSc by using abatacept (an immunosuppressive agent that blocks the interaction of CD28 on naive T cells with CD80 and CD86 on antigen-presenting cells), which is the subject of the ASSET clinical trial in SSc[8]. They noted that abatacept diminished the accumulation of $CD4^+$ CTLs in the skin of three of the four patients with active SSc from the ASSET trial who were tested[2], suggesting that abatacept treatment might block the differentiation of naive and memory $CD4^+$ T cells, and potentially $CD8^+$ T cells, into effector cells through interference with co-stimulation. This result provides a potential rationale for treating patients with early SSc with interventions that prevent T cell activation or attenuate T cell cytotoxicity.

Lastly, Maehara et al.[2] suggest that the accumulation of apoptotic cells in SSc tissue resulting from CTL killing might contribute to tissue damage and remodelling processes that lead to tissue fibrosis, consistent with the prominent vasculopathy that precedes fibrosis. Indeed, apoptotic cells facilitate wound healing by macrophages[9] and contribute to the fibrotic process in idiopathic pulmonary fibrosis[10]. In addition to their cytotoxic function, Maehara et al. showed that $CD4^+$ CTLs produce the pro-inflammatory and pro-fibrotic cytokine IL-1β and found a positive correlation between $CD4^+$ CTL expansion and the number of activated myofibroblasts in the skin samples. Similarly, circulating and skin-resident $CD8^+$ T cells from patients with early dcSSc produce high concentrations of the pro-fibrotic cytokine IL-13, which correlates with the extent of skin fibrosis and induces a pro-fibrotic phenotype in normal fibroblasts and fibroblasts from patients with SSc in vitro[4,5].

Collectively, all these findings provide new insights into the pathogenesis of SSc by indicating that cytotoxic T cells in SSc skin lesions might contribute to vasculopathy and tissue fibrosis through targeted endothelial cell killing and by secreting pro-inflammatory and pro-fibrotic cytokines that lead to abnormal tissue remodelling, consequently compromising tissue and organ function. A model describing the current knowledge of the role of cytotoxic T cells in the pathogenesis of dcSSc is presented in FIG. 1. The study by Maehara et al.[2] provides a rationale for targeting the activation of CTLs or their specific effector functions when developing innovative therapies that might result in better efficacy and less toxicity than existing therapies for this currently incurable disease. Future studies are needed to confirm these results in a larger cohort of patients, and it is hoped that a deeper understanding of the molecular mechanisms will lead to more effective and personalized therapeutic approaches.

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Competing interests

The author declares no competing interests.

Protecting the kidney in systemic lupus erythematosus: from diagnosis to therapy

Naomi I. Maria and Anne Davidson ✉

Abstract | Lupus nephritis (LN) is a common manifestation of systemic lupus erythematosus that can lead to irreversible renal impairment. Although the prognosis of LN has improved substantially over the past 50 years, outcomes have plateaued in the USA in the past 20 years as immunosuppressive therapies have failed to reverse disease in more than half of treated patients. This failure might reflect disease complexity and heterogeneity, as well as social and economic barriers to health-care access that can delay intervention until after damage has already occurred. LN progression is still poorly understood and involves multiple cell types and both immune and non-immune mechanisms. Single-cell analysis of intrinsic renal cells and infiltrating cells from patients with LN is a new approach that will help to define the pathways of renal injury at a cellular level. Although many new immune-modulating therapies are being tested in the clinic, the development of therapies to improve regeneration of the injured kidney and to prevent fibrosis requires a better understanding of the mechanisms of LN progression. This mechanistic understanding, together with the development of clinical measures to evaluate risk and detect early disease and better access to expert health-care providers, should improve outcomes for patients with LN.

Capillary rarefaction
A loss of capillary structure leading to reduced density of microvascular networks.

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Lupus nephritis (LN) affects up to 40% of adults and 80% of children with systemic lupus erythematosus (SLE) and is a major cause of morbidity and mortality[1,2]. LN occurs most frequently and is most severe in adolescents, in patients of non-European ancestry and in patients of lower socioeconomic status[3]. Current standard-of-care therapy comprises induction therapy with high-dose immunosuppressants and glucocorticoids followed by a maintenance phase that lasts for several years and then the gradual withdrawal of therapy[4,5]. However, even within the setting of clinical trials, remission is achieved in only 30–50% of patients, and 10–20% of patients develop end-stage renal disease (ESRD) within 10 years of diagnosis[3,6]. Although reported improvements in LN outcomes have been attributed to earlier diagnosis and optimal management in European patients over the past decade[7], the risk of ESRD has not improved in the USA since the late 2000s[3,8]. This lack of improvement is partly due to the substantial barriers imposed by the poor access faced by many patients with LN in the USA to high-quality health care and incomplete adherence to treatment regimens[9,10].

In general, improvement in outcomes for patients with LN will require both new knowledge and new strategies for conducting clinical trials. Rapid progress in our understanding of the immune mechanisms involved in LN is leading to the design of new immunosuppressive drugs with defined targets and improved safety profiles. Similarly, a better understanding of the non-immune mechanisms of renal injury and repair could yield new ways of preventing the progression of chronic kidney disease (CKD). Substantial heterogeneity exists among patients, a difficulty that could be addressed by the improved use of biomarkers in diagnosis and by selecting patients for clinical trials on the basis of which pathogenic mechanisms are involved in promoting their disease. In this Review, we provide an overview of mechanisms of renal damage in LN, summarize the role of novel technologies in providing new data and address how such information might be exploited to achieve diagnostic and therapeutic advances for patients with LN.

The pathogenesis of lupus nephritis

LN is initiated by the deposition of nucleic acid-containing material in the glomeruli, which triggers the engagement of complement, the activation of renal stromal cells and the recruitment of circulating pro-inflammatory cells[11]. Disease progression is associated with tubulointerstitial hypoxia, metabolic dysfunction of the tubular epithelium, tubulointerstitial capillary rarefaction, accumulation of mixed lymphoid

Key points

- Lupus nephritis (LN) is a heterogeneous complication of systemic lupus erythematosus that remains a considerable unmet medical need.
- Genetic and epigenetic factors confer risks of LN incidence and progression.
- Single-cell analyses and enhanced microscopic analyses of renal tissues are yielding new information about LN pathogenesis and the progression of chronic kidney disease.
- Improvements in risk assessment using genetic or transcriptomic biomarkers could enable the design of clinical trials to prevent LN onset and progression.
- Trials might need to be tailored according to the genetic profile of the patient, a biomarker-based evaluation of their renal tissue and/or the mechanism of action of each new drug.
- Developments in the understanding of tubulointerstitial injury and repair are yielding new strategies for preserving renal function and preventing fibrosis.

infiltrates and fibrosis (FIG. 1). Distinguishing features of LN include the high degree of associated systemic inflammation, the deposition of immune complexes that contain ligands for endosomal Toll-like receptors (TLRs), the activation of inflammasome-mediated and type I interferon-mediated pathways that contribute to endothelial dysfunction[12,13], the production of pathogenic antibodies to complement protein C1q that amplify complement-mediated injury[14] and an additional tendency towards thrombosis.

Genetic risk of lupus nephritis

Genetic polymorphisms contribute to both the loss of immune tolerance that precedes the development of pathogenic autoantibodies and the risk of development and progression of LN (FIG. 1a). More than 100 genetic polymorphisms are associated with the risk of developing SLE, many of which are linked to myeloid cell and B cell activation pathways and the type I interferon pathway[15,16]. Several of these genetic risk alleles, including HLA alleles and the newly identified *BAFF* variant[17], correlate with the early onset of both SLE and LN, suggesting that some SLE risk polymorphisms also predispose to LN[18]. By contrast, some genetic polymorphisms, such as *PDGFRA*[19], are associated with LN risk but not with SLE risk per se; in this context, differences have been observed between individuals of different ethnic backgrounds[20]. Finally, some gene polymorphisms are associated with the risk of progression to CKD in patients with underlying renal disease of any cause[21,22]. Of these, the *APOL1* polymorphism that is associated with CKD progression in African Americans might partially be responsible for poorer LN outcomes in African-American patients as it is associated with both an increased risk of LN and a more rapid disease progression[23]. Functional analysis of LN risk genes has revealed various possible pathogenic mechanisms, including induction of a pro-inflammatory state (*ITGAM*, *FCGR3A*, *TNIP1*, *TNFSF4*, *IRF5* and *NFATC*), altered immune complex clearance (*FCGR2A*) and altered intrinsic response to renal injury (*APOL1*, *DAB2*, *PDGFRA*, *KLK* and *HAS2*)[24]. At the individual level, each polymorphism contributes only a small increase to the odds ratio for LN, making it difficult to reliably predict LN risk on the basis of genetic testing.

Glomerular injury

Initial immune complex-mediated glomerular damage varies according to the site of immune complex deposition (FIG. 1b). Subendothelial deposits cause the recruitment of pro-inflammatory cells from the blood, leading to proliferative disease and glomerular crescents, whereas subepithelial deposits that contact only the urinary space cause membranous disease, characterized by podocyte injury with foot process effacement and consequent proteinuria. Podocytes, endothelial cells and mesangial cells within the glomerulus interact with and support each other: podocytes produce vascular endothelial growth factor (VEGF) and other angiogenic factors required for endothelial cell survival[25,26]; endothelial cells make platelet-derived growth factor (PDGF) that is needed for mesangial cell survival; and mesangial cells sequester latent transforming growth factor-β (TGFβ), thereby protecting the endothelium from apoptosis[27]. Therefore, progressive injury to one cell type can eventually lead to damage of the other cell types.

Activation, dedifferentiation or proliferation of glomerular cells causes loss of structural integrity to the glomerular tuft and eventual nephron death. Glomerular endothelial cells are also damaged by circulating pro-inflammatory mediators and by TLR ligand-mediated activation, which induces the release of cytokines that cause glomerular cell death and of chemokines that enhance the recruitment of circulating immune cells[28,29]. Injured glomerular cells amplify damage and inflammation by a variety of mechanisms. Damaged podocytes and endothelial cells both secrete endothelin 1, which causes vasoconstriction and mitochondrial dysfunction[25]. Stressed endothelial cells also release pro-inflammatory and pro-coagulant mediators and increase their expression of adhesion molecules such as VCAM1 and ICAM1, which aid the recruitment of circulating immune cells[25]. Podocytes and mesangial cells further amplify inflammation by producing pro-inflammatory cytokines, such as IL-6 and IL-1, chemokines and growth factors, including macrophage colony-stimulating factor (M-CSF)[27,30–32].

Both mesangial cells and podocytes have limited regenerative capacity, and their loss is associated with glomerulosclerosis[33,34]. Therefore, methods for the early detection of glomerular injury are needed so that therapies that preserve glomerular structure and function in patients with LN can be used. For example, in lupus-prone MRL/*lpr* mice, increased podocyte expression of calcium/calmodulin-dependent protein kinase type IV (CAMK4), a protein that regulates podocyte integrity, precedes the onset of proteinuria. In these mice, podocyte-targeted delivery of an inhibitor of CAMK4 during the time window between increased expression and proteinuria onset protected podocytes from toxic injury and foot process effacement[35]. However, this type of intervention depends on disease stage and might not be effective once podocytes have been lost. An important challenge for the treatment of human LN will be to identify targets that are early mediators of injury and to understand at what stages of disease early-acting therapies should be instituted to prevent irreversible renal damage.

Glomerular crescents
A response to severe injury in which crescent-shaped glomerular lesions that consist of epithelial cells, fibroblasts, immune cells and matrix form adjacent to the Bowman's capsule.

Foot process effacement
A podocyte reaction to injury or damage in which the epithelial foot processes become flattened and lose their barrier function, resulting in proteinuria.

Glomerular tuft
A network of small blood vessels and supporting cells that forms the initial structural component of the nephron.

Glomerulosclerosis
Scarring of the glomeruli that leads to loss of function.

Although glomerular injury in LN is classically initiated by glomerular immune complex deposition, a rare form of LN that also injures the glomeruli is thrombotic microangiopathy, in which complement-mediated endothelial injury causes glomerular microthrombi that are associated with proteinuria, haemolytic anaemia, thrombocytopenia, hypertension and rapidly declining renal function. Thrombotic microangiopathy is related to other complement-mediated thrombotic renal diseases such as haemolytic uraemic syndrome and can be successfully treated with complement protein C5 inhibition[36].

Tubulointerstitial injury

The blood supply to the renal tubulointerstitium is provided by run-off from the glomeruli; therefore, glomerular loss compromises tubulointerstitial viability. Changes to the renal tubulointerstitium caused by this loss in viability, such as tubular atrophy, fibrosis and interstitial infiltrates (FIG. 1c), are known prognostic

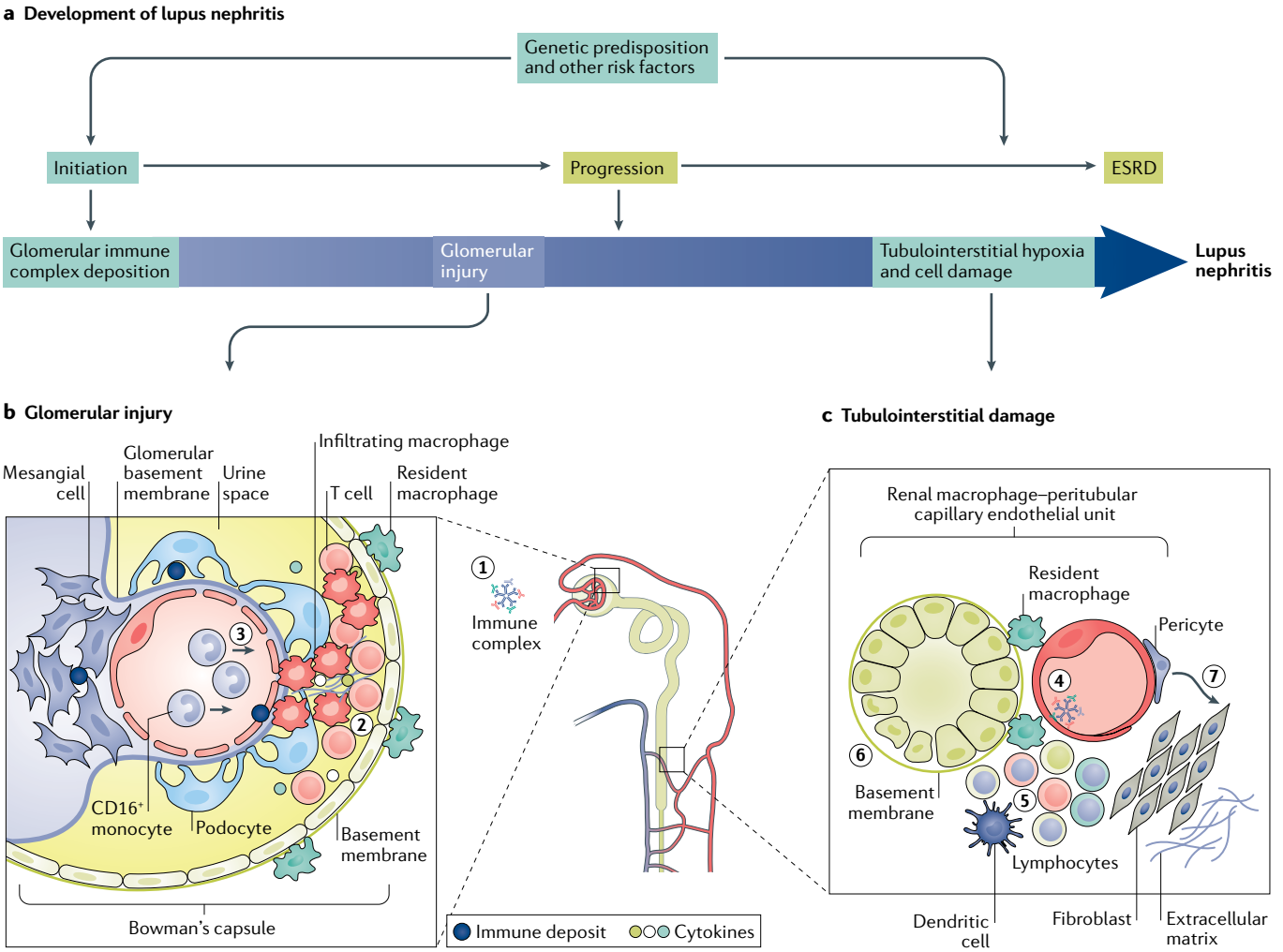

Fig. 1 | **Glomerular injury and tubulointerstitial damage in lupus nephritis. a** | Schematic timeline representing the development and progression of lupus nephritis. Genetic polymorphisms and other risk factors contribute to both the initiation phase of glomerular injury and the risk of subsequent tubulointerstitial damage during the development of lupus nephritis. **b** | During glomerular injury, circulating pro-inflammatory cytokines and the subendothelial deposition of immune complexes (1) contribute to endothelial dysfunction and the recruitment of pro-inflammatory CD16$^+$ macrophages and T cells into crescents (2) that might also contain proliferating epithelial cells from the parietal layer of the Bowman's capsule. CD16$^+$ monocytes are recruited from the blood into the crescents (3), and changes in their gene expression profiles occur as they begin to infiltrate into the tissue parenchyma and differentiate into macrophages. Resident renal macrophages are located around the outside of the Bowman's capsule, where new lymphoid tissue often accumulates during chronic inflammation. Subepithelial and mesangial deposition of immune complexes causes damage to podocytes and mesangial cells, respectively, but pro-inflammatory cell recruitment to these sites is limited during glomerular injury because the cells have little access to the intravascular space. **c** | Resident renal macrophages are located next to tubules and tubular capillaries in the 'renal macrophage–peritubular capillary endothelial unit'. Renal macrophages can be activated by small immune complexes that transit from the adjacent endothelium to resident macrophages owing to the lack of a basement membrane (4). Recruited tubulointerstitial pro-inflammatory immune cell infiltrates include myeloid dendritic cells, plasmacytoid dendritic cells and various lymphocytes and are sites at which antigen presentation to T cells and T cell–B cell interactions can promote the differentiation of B cells into plasma cells that secrete antibodies to renal antigens (5). Inflammation and tubulointerstitial hypoxia induce metabolic dysfunction and atrophy of tubular cells (6) with inadequate repair. Growth factors such as transforming growth factor-β induce fibroblast differentiation from mesenchymal stromal cells such as pericytes, resulting in renal fibrosis and irreversible damage (7). ESRD, end-stage renal disease.

Fate mapping
A technique used in developmental biology to study the embryonic origin of adult cells, tissues and structures.

markers for CKD progression in patients with LN[37]. Tubular epithelial cell injury is an important cause of renal fibrosis[38], although fate mapping studies have cast doubt on the ability of tubular epithelial cells themselves to directly transition to myofibroblasts[39,40]. One potential mechanism is the secretion, by tubular epithelial cells, of pro-fibrotic factors that activate tubulointerstitial pericytes. These cells are embedded into the basement membrane of small peritubular vessels; pericytes can differentiate into myofibroblasts in injured kidneys[39,41] and can mediate pro-inflammatory signalling via a MyD88-dependent mechanism[39]. Other mesenchymal stromal cells can also differentiate into fibroblasts, often via the transcription factor MYC, which is important in promoting this process[42]. Detachment of activated pericytes from the endothelium leads to capillary rarefaction, which can be irreversible. Capillary loss also results from attenuated production of VEGF by hypoxic tubular epithelial cells[43].

Small immune complexes that are cleared through interstitial capillaries are taken up by adjacent interstitial resident macrophages; engagement of the Fc receptor FcγRIV and endosomal TLR pathways synergistically activate these cells when they encounter immune complexes containing nucleic acids[44]. Resident macrophages, together with activated fibroblasts, contribute to renal injury by secreting pro-inflammatory mediators that attract immune cells to the interstitium[39], a feature that is associated with worse outcomes in patients with LN[45].

Immune cells in lupus nephritis

Glomerular infiltrates in LN consist mainly of macrophages, with T cells present in the more severe crescentic forms[46,47]. Glomerular macrophages are recruited from the pool of circulating monocytes and have been extensively studied in mouse models of LN. Endothelial cells that are activated via nucleic acid-sensing TLRs such as TLR7 preferentially recruit patrolling monocytes[28], a $CD11c^{+}Ly6C^{lo}$ population characterized by the transcription factor Nr4a1. Glomerular $CD11c^{+}$ cells have been uniformly observed in mouse models of LN in which TLR7 is overexpressed[48–50], and the human equivalent ($CD16^{+}$ monocytes), rather than the pro-inflammatory $CD14^{+}$ monocyte population, are preferentially recruited to human LN tissue[51]. Excessive intrinsic endosomal TLR signalling in monocytes results in glomerular recruitment of patrolling monocytes, leading to the subsequent recruitment of neutrophils, which cause glomerular damage even in the absence of serum autoantibodies[52]. Taken together, these studies[48–50] identify the activation of TLRs in both the renal endothelium and circulating monocytes as important factors in recruiting pathogenic $Ly6C^{lo}$ monocytes to glomeruli in LN. However, this mechanism might not be the only way in which glomerular macrophages are recruited, as a glomerular population of $F4/80^{lo}CD11c^{lo}$ alternatively activated macrophages has also been described in NZM2328 mice[53]. Classical inflammatory $Ly6C^{hi}$ macrophages have been reported in only a few mouse models of lupus, although they are a prominent feature of ischaemic and anti-glomerular basement membrane-mediated glomerular injury.

Mixed tubulointerstitial leukocyte infiltrates, sometimes with features of lymphoid organization, are found in many forms of CKD, including LN[45,54–56]. The immune responses that occur in situ during progressive LN have been examined using single-cell analyses (see the Single-cell analysis section below) and by mapping cellular position and shape within a tissue to identify cognate interactions. B cell and T cell clones are present in LN tissue, and T helper cells that express high amounts of inducible T cell costimulator (ICOS) and IL-21 are located next to B cells in the renal infiltrates[57–59]. The presence of antigen-presenting cells, such as myeloid dendritic cells (DCs) and plasmacytoid DCs[59,60], is associated with more advanced disease in LN[61]. Renal B cell responses are dominated by reactivity to the intrinsic renal antigen vimentin, an intermediate filament protein that is aberrantly released from injured cells[62]. Autoantibodies to vimentin can occur following any renal transplantation and are linked to allograft injury, but the clinical utility of measuring these antibodies in LN is not known[63].

In addition to infiltrating cells, the kidneys have a network of tissue-resident macrophages located around glomeruli and in the tubular interstitium that are involved in immune surveillance[64,65]. Peritubular renal macrophages are particularly susceptible to immune complex-mediated activation owing to their anatomical location near to small peritubular vessels that lack an intervening basement membrane[44] (FIG. 1c). Interestingly, an increase in the number of tissue-resident macrophages that express genes related to both pro-inflammatory and pro-reparative features has been reported in mouse models of LN[43,66], suggesting either a dysregulated repair process or the presence of more than one cell subpopulation.

Overall, many types of immune cells are found in the kidneys of individuals with LN. A better understanding of how each infiltrating cell type contributes to renal injury is now needed so that pathogenic cells can be targeted, whereas those involved in organ protection and repair can be spared. In particular, the role of macrophages with a reparative phenotype is not well defined. These cells are required to prevent fibrosis after acute renal inflammation, but can become dysregulated and promote tissue injury during chronic inflammation[67].

Diagnosis and monitoring

A diagnosis of LN is currently made when there is a change in the clinical status of a patient, such as haematuria, proteinuria or a decline in renal function, which prompts a confirmatory renal biopsy. The tissue sample is graded histopathologically according to the International Society of Nephrology–Renal Pathology Society (ISN–RPS) classification, which includes indices for active inflammation and chronicity[68]. The presence of tubulointerstitial inflammatory cell infiltrates and a high chronicity index are both associated with a worse prognosis, independent of glomerular changes[37,45]. Nevertheless, analysis of renal tissue is not always an accurate indicator of renal outcome, and samples taken by biopsy from patients in full clinical remission can show ongoing inflammation[69]. Controversy exists as to

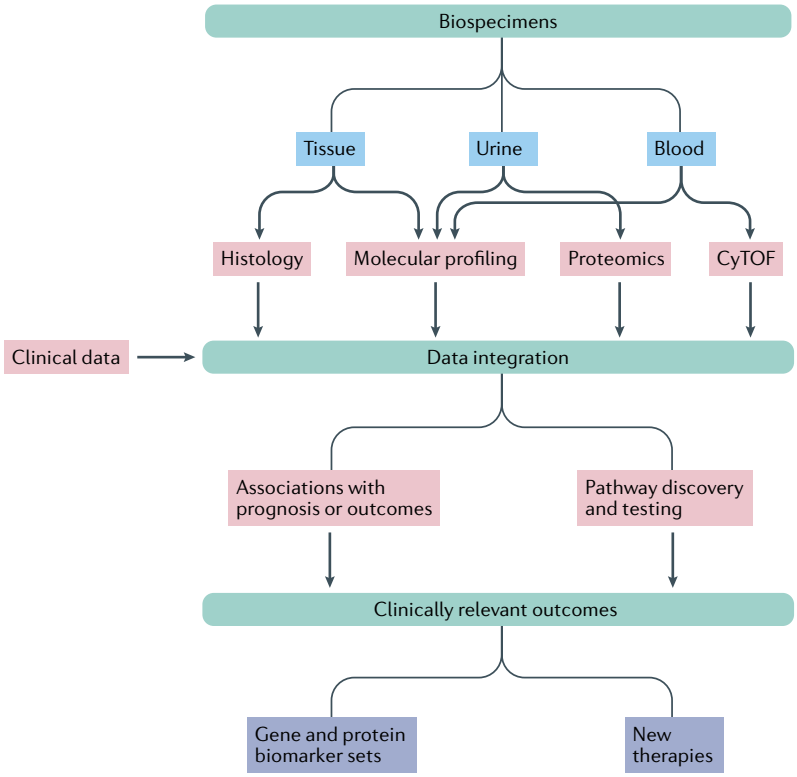

Fig. 2 | **Data integration for lupus nephritis diagnosis and therapy.** Clinical data from patients with lupus nephritis can be integrated with phenotypic, molecular and proteomic data obtained from biospecimens to predict the prognosis and response to treatment of these patients and to discover new pathogenic pathways for downstream testing. Big data analysis will benefit from the further development of machine learning and bioinformatics algorithms. These data-driven results should produce clinically relevant outcomes such as biomarker sets (both gene and protein) and new patient-specific therapies. CyTOF, cytometry by time-of-flight.

whether escalating therapy in patients with evidence of residual inflammation in such a follow-up tissue sample will improve renal outcome[69,70] and, to date, a decrease in proteinuria to <0.7–0.8 g/dl by 1 year after diagnosis is the best predictor of long-term outcome[71,72].

Biomarker analyses

Given that renal histology might not be predictive of LN outcome, meeting the need for effective therapies in LN requires biomarkers for disease risk, as well as for response to therapy. Such biomarkers could include changes in circulating cells, concentrations of inflammatory mediators, specific urinary proteins or molecular signatures that can be assessed in renal tissue. New discovery-based approaches should lead to better diagnostic and therapeutic strategies for LN (FIG. 2).

Serum and urine biomarkers. A number of urine biomarkers can be used to differentiate patients with active LN from those with inactive disease, and multiplexed approaches have been used to identify panels of biomarkers that are associated with LN in cross-sectional studies[73–75]. However, no new biomarkers have yet been shown to outperform the estimated glomerular filtration rate or proteinuria as measures for diagnosis, and none is currently used in clinical practice. Few studies have as yet addressed whether longitudinal biomarker analyses can predict LN risk, detect LN before renal injury becomes clinically evident or identify those patients at highest risk of subsequent renal decline after an initial flare[73]. An increase in plasma concentrations of soluble urokinase-type plasminogen activator receptor (uPAR) and a decrease in the urinary epidermal growth factor (EGF)–creatinine ratio are independent predictors of progression to CKD in patients with glomerular disease of multiple aetiologies[76,77]. However, these biomarkers have not yet been systematically applied to the longitudinal study of patients with LN. The application of new proteomic technologies for unbiased and sensitive multiplexing of multiple markers will address whether it is possible to differentiate between ISN–RPS histological classes, identify treatment responders or predict long-term outcomes from serum or urine alone[78].

Modular signatures. Molecular approaches have been used to identify peripheral blood cell signatures that are associated with an increased risk of LN or SLE flares[79,80]. One approach to simplifying the analysis of large data sets such as the whole-blood transcriptome is modular repertoire analysis, a computer-based algorithmic approach that uses a modular transcriptional analysis framework for transcriptomic studies. Modules are defined on the basis of a co-expression matrix (a module corresponds to a group of genes that are consistently co-expressed across several data sets). This data-reduction approach can be used to identify distinct, disease-specific modular gene-specific and cell-specific signatures within cross-sectional data sets and to analyse longitudinal data sets in large patient cohorts[80]. Simplified modules based on co-clustered gene sets[81] have been used to probe functional pathways that are abnormally expressed in patients. These studies have demonstrated molecular complexity and heterogeneity among patients with SLE[80,82,83]. Analysis of a large longitudinal data set from a paediatric cohort of patients with SLE identified seven distinct patient clusters distinguished by their molecular profiles and clinical traits. In particular, a 20-gene neutrophil signature was associated with LN in these patients[80] and with either present or past LN in adults with SLE[79,83,84]. However, these studies are complicated by the increase in the number of neutrophils induced by moderate-to-high glucocorticoid doses of >20 mg daily. Furthermore, the application of a nine-gene neutrophil score to longitudinal data failed to show an association with histological disease severity or class, or with the risk of subsequent flare[84]. Reanalysis of the paediatric data, together with a data set from adult patients, identified three molecular clusters that did not change with either disease activity or treatment, of which only one, characterized by a lymphocyte rather than a neutrophil signature, had a significantly decreased risk for LN ($P < 0.05$ compared with the other two clusters)[79]. Further work by the same group suggests that responsiveness of the gene signature to different treatments might also be cluster specific[85]. In a third study of adult patients with SLE, peripheral blood signatures associated with active disease included oxidative phosphorylation, ribosome, proteasome, cell cycle and pyrimidine

Box 1 | **Stratifying patients with lupus nephritis for clinical trials**

Several strategies exist for stratifying patients with lupus nephritis into subgroups for clinical trials, including stratification on the following bases.

Immune mechanism

- Extrafollicular B cell activation is promoted by innate immune mechanisms, whereas germinal centre B cell activation is promoted by T cells. These mechanisms could be targeted in patients with strong innate immune or T cell signatures.
- Enhancing the $CD8^+$ T cell exhaustion signature might help to prevent disease flares.
- Single-cell analyses and cross-species comparisons will foster investigation of new mechanisms and testing of new therapeutic strategies in mouse models chosen to reflect aspects of human disease.

Genetic profile

- Correcting the immune defect conferred by polymorphisms affecting different cell types might be an individualized strategy to maintain disease quiescence.
- Addressing polymorphisms that increase the risk of chronic kidney disease progression might require non-immune-based strategies to protect the kidney.

Biomarkers

- Modular profiling might identify patients at highest risk of lupus nephritis flare who can be enrolled in disease prevention studies.
- Unbiased approaches and modular analyses of peripheral blood could define patient subgroups that can be tested for responsiveness to particular therapies.

Disease stage and histological findings

- Treatment of flare and prevention of flare might each require different therapies, perhaps administered sequentially.
- The study of molecular patterns in renal tissue might yield new predictors of response to therapy and outcome that inform patient selection for trials.
- Tissue samples taken once treatment is underway might identify patients for whom treatment withdrawal is safe.
- The presence of fibrosis might require an adjuvant anti-fibrotic approach.

metabolism pathways, with the addition of a plasmablast signature in patients with LN[86]. By contrast, the plasmablast signature was associated with overall disease activity, but not specifically with LN in paediatric patients with SLE[81].

A different molecular signature identified by analysis of isolated peripheral blood T cells is a combination of a low co-stimulation signature in $CD4^+$ T cells with an exhaustion signature in $CD8^+$ T cells. This signature is associated with a poor response to infection or immunization, but also confers a decreased risk of flares in patients with SLE or anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis[87,88]. The limited data available suggest that assignment of patients to the $CD8^+$ T cell exhaustion phenotype, and even to some of the modular phenotypes described above, is mostly independent of either disease activity or treatment, so that it might be possible to use this type of data to stratify patients for LN risk or to use the LN-associated modules and cluster assignments to direct individualized therapy (BOX 1). However, although it is clear that there is much heterogeneity among patients with SLE, more longitudinal molecular and clinical data will be needed to address these issues.

Exhaustion signature
A cell state or phenotype with progressive loss of effector cytokine or cytotoxic function owing to prolonged antigen stimulation, often characterized by the increased expression of immune checkpoint inhibitory receptors, alterations in metabolic function and a distinct transcriptional profile that differs from that of anergic cells.

Epigenetic profiling. Epigenetic profiling measures biochemically reversible changes in chromatin structure that influence whether the chromatin is accessible for gene transcription and expression of its associated protein. Changes in DNA methylation and in histone methylation and acetylation can be mapped using biochemical methods[89]. For example, epigenome-wide association studies examine the association between gene expression and disease risk by profiling the methylation and acetylation status of DNA and/or histones within peripheral blood mononuclear cells to identify specific genes that are repressed or accessible[89]. Alternatively, assay for transposase-accessible sequencing (ATAC-seq) can be used to identify areas of open chromatin that are potentially available for gene transcription. Epigenetic modifications can be induced by external factors, such as smoking, oxidative stress or medications, but also vary by ethnicity[90,91].

Hypomethylation of type I interferon response genes has been observed in immune cells of most lineages from patients with SLE compared with immune cells from healthy individuals, although this hypomethylation is also found in other autoimmune diseases, such as rheumatoid arthritis and systemic sclerosis[92]. Cross-sectional studies have revealed differences in the methylation status of $CD4^+$ T cells and total peripheral blood mononuclear cells from patients with SLE and LN compared with patients with SLE and no LN, but it is not yet known whether clinically useful data can be derived from such studies[89]. In lupus-prone mice, DNA hypomethylation in T cells caused increased expression of pro-inflammatory genes and induced autoimmunity[93]. However, the targeting of a DNA methylation inhibitor specifically to T cells ameliorated disease in the MRL/*lpr* mouse model of lupus[94], indicating that the relationship between methylation differences and disease expression is complex and that the result of therapeutic targeting cannot be easily predicted.

Omics analyses of the kidneys

New technologies are now enabling molecular, biochemical and cellular analyses to be performed on blood, urine and the small amounts of renal tissue that can be obtained as part of diagnostic tissue sampling in patients with LN[78]. The discussion below looks at how these omics technologies can be used to examine whole organs or single cells.

Whole-organ analyses. Molecular profiling of fibrotic kidney tissue from patients with CKD of multiple aetiologies, including LN, has revealed inflammation and tubular metabolic dysfunction as the most important dysregulated pathways in a disease state[95]. Further analysis showed that kidney tubular epithelial cells depend on fatty acid oxidation and mitochondrial oxidative phosphorylation as their main energy sources, and that these metabolic functions are compromised in progressive CKD, as shown by downregulation of the transcription factor PPARGC1α (an important regulator of mitochondrial biogenesis)[95] and a concomitant switch from oxidative phosphorylation to glycolysis[42]. Proteome screening subsequently indicated that the transcription factors PPARα and PPARγ induce the production of lipid-metabolizing enzymes and suppress that of glycolytic enzymes in the proximal tubules of healthy kidneys[96]. Similarly, screening for microRNA (miRNA)

expression in both human and mouse fibrotic kidneys revealed a role for miR-21 in regulating genes involved in mitochondrial biogenesis and fatty acid oxidation, thus favouring glycolysis in the injured tissues[97].

Tubular epithelial cells that are arrested at the G2–M transition point of the cell cycle fail to proliferate and undergo repair[38,98]. Transcriptome analysis of renal tissue from patients with renal disease of multiple aetiologies, including LN, revealed a panel of 72 genes, the expression of which correlated with the estimated glomerular filtration rate[77]. This panel contained genes involved in tissue remodelling and fibrosis, including *EGF*, which is regulated by PPARγ and has been linked to the regenerative capacity of renal tubules[77,96]. Studies over the past decade have revealed several other pathways that determine whether tubules will undergo regeneration or senescence[99], including a role for the transcription factor FOXO3 in regulating autophagy, which provides lipids for mitochondrial oxidation[100]. Because tubular damage induces renal fibrosis, the translation of these studies to therapeutic approaches is urgently needed.

Mice and humans with LN share common renal molecular signatures[43]. Longitudinal transcriptomic studies of kidneys from NZB/W F1 mice showed an increase in the expression of multiple pro-inflammatory genes at proteinuria onset, followed by the downregulation of a set of PPARGC1α-regulated genes, indicative of metabolic and mitochondrial dysfunction as the mice progressed towards renal failure[101]. This signature can be reversed with remission induction therapy, but metabolic dysfunction recurred before clinical relapse, suggesting that, once initially injured, the kidney becomes more susceptible to damage during subsequent disease flares[101]. These studies further showed that the decreased expression of VEGF at proteinuria onset does not reverse with remission induction, suggesting that the small renal vessels might be compromised, even during remission[43,101]. Preclinical translation of human-relevant findings into therapeutic approaches is beginning. Notably, overexpression of PPARGC1α in kidney tubule cells protects against tubule injury and fibrosis in several mouse models of acute renal injury[95]. Similarly, systemic miR-21 depletion protects against acute renal injury in mice, whereas PPARγ inhibition causes tubulointerstitial fibrosis[96].

Single-cell analyses. Single-cell RNA sequencing is an important technical advance that has enabled the molecular profiling of individual renal cells and immune cells and that should advance our knowledge of the pathogenic mechanisms in LN[102,103]. The advantage of this approach is that rare cell types can be identified and differences in gene expression profiles between cells of each renal and infiltrating cell type from healthy individuals and patients with LN can be evaluated. The Accelerating Medicines Partnership (AMP) is currently undertaking a large-scale project in LN; enrolment is completed and analysis is planned for 160–200 tissue samples from patients with LN that have associated clinical metadata, peripheral blood analyses and urine proteomic data[78]. Molecular and proteomic analyses of renal tissue and urine will be correlated with disease outcomes at 12 months and have the potential to reveal additional biomarkers for treatment response and outcome[78].

Initial results of single-cell data from the first phase of the AMP LN studies were published in 2019 (REFS[51,104]). Preliminary analyses of tubular epithelial cells from 21 patients with LN suggested that patients with proliferative disease or who were unresponsive to 6 months of treatment had a higher baseline interferon signature[104]. Treatment non-response was also associated with a tissue remodelling or fibrosis signature that was independent of histologically evident fibrosis[104]. Single-cell RNA sequencing analysis of immune cells sorted from kidney samples from 24 patients with LN and ten living transplant donors revealed multiple immune cell types in the LN tissues, including ten clusters of natural killer cells and T cells, four clusters of B cells, six clusters of macrophages and DCs and one mixed cluster of dividing cells[51]. By contrast, the immune cell populations in healthy kidney were less diverse and dominated by memory $CD4^+$ T cells and resident macrophages. Apart from the confirmation of in situ B cell activation and differentiation, several novel observations have come from this study[51], including the identification of an interferon signature in most cell types; the presence of proliferating cells consisting mostly of natural killer cells and $CD8^+$ T cells; the identification of novel $CD8^+$ T cell subsets; the absence of exhausted $CD8^+$ T cells; and a lack of clear skewing of $CD4^+$ T cells to either a T helper 1 cell or T helper 17 cell phenotype. Renally derived leukocytes could also be detected in the urine, suggesting the possibility of non-invasive profiling[85].

Focusing on myeloid cells, the infiltrates in the kidneys of patients with LN included both classic and plasmacytoid DCs, as well as four subpopulations of macrophages or monocytes, only one of which could be detected in the peripheral blood[51]. One of these subsets was also detected in healthy kidney, and therefore probably represents a tissue-resident renal macrophage population; however, the transcriptome of this macrophage population is modulated in LN, whereby it acquires a mixed interferon signature and an anti-inflammatory signature. The other three macrophage or monocyte subpopulations are related along a developmental trajectory that starts with a population that most resembles pro-inflammatory $CD16^+$ monocytes and progresses to a phagocytic macrophage phenotype and then to an alternatively activated phenotype that is also a major cellular source of chemokines[51]. The presence of $CD16^+$ monocytes in the kidneys of patients with LN is consistent with previous histological data[105,106] and with results in mouse models of LN in which $Ly6c^{lo}$ monocytes are recruited to the kidneys[48]. The reason for the preferential recruitment of the $CD16^+$ monocyte population over the $CD14^+$ population in LN could reflect the important role of TLR activation by nucleic acid-containing immune complexes and debris in the recruitment of patrolling monocytes.

Overall, these studies[51,104] have established the feasibility of applying a single-cell analysis approach to LN and have highlighted the complexity and heterogeneity of the intra-renal immune response that contributes to disease progression. Data from the phase II AMP studies

that are currently in progress will enable the robust correlation of molecular patterns with outcomes, as well as the discovery of new pathogenic mechanisms that might inform us about how best to therapeutically target pathogenic immune cell populations and modulate stromal cell dysfunction in LN. Given that changes in the tubular epithelium are associated with progression and fibrosis, it will be of great interest to determine whether signatures of tubular regenerative capacity and metabolic function can be inferred from the molecular data and correlated with disease outcome.

Future perspectives for therapy

Standard immunosuppressive treatments for LN have a high non-response rate and currently, only one biologic drug, belimumab, is approved for the treatment of SLE, and none for the treatment of LN. However, this poor outlook could be about to change.

Improving clinical trial design

The failure of clinical trials to yield an effective new treatment strategy for LN has partly been attributed to the complexities of clinical trial design[107,108]. LN is a heterogeneous disease, and the global recruitment of patients to clinical trials adds additional geographical diversity in ethnicity, approaches to standard of care and the risk of adverse events. All new drugs are tested against a background of standard-of-care therapy that confers a response rate higher than that of a typical placebo response, necessitating large cohorts to observe meaningful differences. Another difficult issue in LN clinical trial design is how to mitigate the confounding effects of glucocorticoids and standard-of-care therapies, the potential interactions of which with any new drug are mostly unknown. Comparisons between completed trials are also challenging because of differences in design and outcome measures.

Lessons from failed clinical trials in LN[109] have spurred efforts to define the most informative outcome measures and to consider the addition of molecular phenotyping. In this regard, a proteinuria measurement of <0.7–0.8 g/dl at 12 months needs to be tested as a surrogate biomarker for long-term outcome in patients with LN. Because the immune disturbances underlying disease initiation are heterogeneous, individualized approaches might be required to target the inciting cells or pathways; strategies for this approach are outlined in BOX 1. Accurate evaluation of risk in individual patients is needed so that smaller and shorter preventive trials in high-risk patients can be performed without unnecessary exposure of low-risk patients to potentially toxic regimens. In addition, reliable and inexpensive biomarkers for the early stages of LN or of LN recurrence need to be identified so that all patients can be monitored more closely and treated earlier. Finally, because patient compliance with polypharmacy is low, methods for monitoring and encouraging compliance need to be built into clinical trials and clinical practice. The establishment of LN registries that contain both clinical data and biospecimens and the universal access of patients with LN to specialized care and clinical trials will accelerate progress in all areas.

Polypharmacy
The use of multiple medications to treat complex medical conditions.

New therapeutic approaches

New strategies to prevent and treat LN continue to be reported in mouse models (TABLE 1), but these studies often use a single model of disease and do not have standardized intervention times or outcome measures. The failure to translate many therapies that are effective in mouse models of LN into successful clinical trials in patients with LN possibly reflects not only interspecies differences but also the relatively late detection of human disease and the heterogeneity of renal injury mechanisms in genetically diverse human populations. Despite these setbacks, ongoing discovery has fuelled continued efforts to repurpose and combine currently available immunosuppressive drugs and to test new immune-modulating therapies.

New successes in immune modulation for the treatment of SLE, as well as LN, seem to be on the horizon. The first of these, a phase III study of belimumab for LN, has achieved its primary end point and all major secondary end points[110] and is on track for regulatory submission during the first half of 2020. Several new late-phase trials in SLE have achieved their primary end point, the most notable of which are a phase II study of the IL-12–IL-23 inhibitor ustekinumab[111] and one of two phase III studies of the type I interferon receptor antagonist anifrolumab[112,113]. A phase II study of anifrolumab for LN is in progress[114], with an estimated completion date of January 2021. In patients with LN, the combination of mycophenolate mofetil (MMF) with the calcineurin antagonist tacrolimus[115] showed improved rates of remission induction over either drug alone in several Asian cohorts[116], although the potential toxicity of this combination[117] has relegated its use in the USA to patients in whom therapy with MMF alone fails. Two successful phase II trials of voclosporin, a potent and safer alternative calcineurin antagonist, in combination with MMF and a rapid steroid taper have also been completed in patients with LN[117,118], and a phase III study has completed recruitment[119]. The phase II trials showed a substantially better response rate to combination treatment than to MMF alone, but complete response rates were still <50%, and adverse events were more common in those receiving combination therapy[117,118]. Finally, the potent B cell-depleting agent obinutuzumab has achieved FDA breakthrough status for expeditious development and review[120] on the basis of a successful phase II study in LN[121]. Other clinical trials in progress for LN that focus on immune modulation as a strategy are listed in TABLE 2.

In addition to the immune-modulating therapies that are currently being tested, a better understanding of the pathways that are associated with renal repair versus progression to CKD is needed so that the non-immune response to renal injury can be modulated, intrinsic renal cells and structure preserved and fibrosis prevented. Although there are, as yet, no approved treatments that halt CKD progression, headway has been made in understanding how to preserve renal tubular cells or how to promote their reparative programmes. Potential future strategies include the modulation of mitochondrial function[39,102], the modulation of miRNAs[97,102], the reversal of tubular cell senescence[102,122],

Table 1 | **New therapeutic strategies for lupus nephritis tested in mouse models of disease**

Targets	Results	Take-home message	Refs
Effector cytokines			
IL-17	MRL/*lpr* IL-17A KO mice and NZB/W mice treated with anti-IL-17A and anti-IFNγ mAbs; only anti-IFNγ mAb improved outcomes in NZB/W mice	IL-17A is not a promising target for LN	130
IL-34	LN and systemic illness is suppressed in IL-34-deficient MRL/*lpr* mice	Intra-renal and systemic IL-34 promotes LN in MRL/*lpr* mice	131
CD40	CD40 antagonist restores glomerular morphology in NZB/W and MRL/*lpr* mice	CD40 blockade induces very robust prevention and treatment of LN	132
JAK	The JAK inhibitor tofacitinib ameliorates LN and inhibits vascular dysfunction in MRL/*lpr* mice	Tofacitinib shows preventive and therapeutic efficacy	133
SYK	Tyrosine protein kinase SYK inhibitor delays LN in NZB/W mice	SYK inhibition shows dose-dependent preventive and therapeutic efficacy	134–136
BTK	BTK-specific inhibition prevents and treats LN in NZB/W and MRL/*lpr* mice, and in the IFNα-induced model	BTK inhibition shows dose-dependent preventive and therapeutic efficacy	137,138
ADAM17	Pharmacological blockade of either TNF or EGFR signalling protected *Fcgr2b*$^{-/-}$ mice from severe renal damage	Inactive rhomboid protein 2–ADAM17-dependent TNF and EGFR signalling promotes LN	139
Inflammasome	A PIM1 inhibitor suppressed NLRP3 inflammasome activation and reduced LN; by contrast, NLRP3 and ASC deficiency worsen disease in C57BL/6^{lpr} mice[140]	The inflammasome is a potential target but has both pathogenic and protective properties	141
	Piperine[a] ameliorated LN in pristane-injected mice through NLRP3 inflammasome inhibition		142
Metabolism			
mTOR	Baicalin[b] ameliorated LN in MRL/*lpr* mice through mTOR axis inhibition	Targeting cell metabolism by inhibition of mTOR might be beneficial for LN	143
	Mangiferin[c] ameliorated LN in FasL-deficient B6/*gld* mice by inducing regulatory T cells via mTOR pathway inhibition		144
	The mTOR inhibitor rapamycin is cytoprotective in podocyte injury[d]		145
	Rapamycin reduces renal fibrosis in NZB/W mice		146
Oxidative phosphorylation and glycolysis	Combined oxidative phosphorylation and glycolysis inhibition by metformin and 2DG reduced disease severity and reversed LN in NZB/W mice	Each therapy individually prevented disease, but both were needed to reverse established disease	147
Kidney cells			
Podocyte CAMK4	Podocyte-targeted CAMK4 inhibition preserved ultrastructure, averted immune complex deposition and crescent formation, and suppressed proteinuria in lupus-prone mice	Targeted CAMK4 inhibition preserves podocyte structure and function when used as preventive therapy	148
Cholinesterase	The cholinesterase inhibitor galantamine attenuates hypertension, glomerulosclerosis and fibrosis in nephritic NZB/W mice	Anti-inflammatory, antihypertensive and renoprotective effects are mediated through the cholinergic anti-inflammatory pathway	149
Other			
DNA methylation	The demethylation inhibitor 5-azacytidine targeting either CD4$^+$ or CD8$^+$ T cells prevents disease in MRL/*lpr* mice by expanding regulatory T cells and inhibiting the expansion of double-negative T cells	Illustrates the complexity of targeting DNA methylation in vivo, as regulatory elements might be hypermethylated	94
Immune cell modulation	MSCs prevent disease in MRL/*lpr* mice in a CCL2-dependent manner	This study is one of many reports of the efficacy of MSCs, which function via various mechanisms	150

2DG, 2-deoxy-D-glucose; ADAM17, disintegrin and metalloproteinase domain-containing protein 17; ASC, apoptosis-associated speck-like protein containing a CARD; BTK, Bruton tyrosine kinase; CAMK4, calcium/calmodulin-dependent protein kinase type IV; CCL2, CC chemokine ligand 2; EGFR, epidermal growth factor receptor; FasL, Fas ligand; JAK, Janus kinase; KO, knockout; LN, lupus nephritis; mAbs, monoclonal antibodies; MSCs, mesenchymal stem cells; mTOR, mechanistic target of rapamycin; NLRP3, NOD-, LRR- and pyrin domain-containing 3; PIM1, serine/threonine-protein kinase pim-1. [a]Piperine is a natural compound in black pepper and related herbs. [b]Baicalin is extracted from an anti-inflammatory traditional Chinese herbal medicine. [c]Mangiferin is extracted from natural herbs, including *Mangifera indica*. [d]Only tested in vitro.

protection of the renal vasculature from injury[25,26,123] and the use of inhibitors of prolyl hydroxygenase (currently being tested for CKD-associated anaemia) that enhance hypoxia-inducible factor (HIF) and FOXO3 activity[99,100] by altering their prolyl hydroxylation and degradation. Pro-fibrotic cytokines such as TGFβ and connective tissue growth factor (CTGF) are also logical therapeutic targets for late-stage disease[39]. The multiple roles of TGFβ in promoting renal fibrosis have been well described, but inhibition of the cytokine itself has not been successful; alternative approaches to targeting TGFβ are reviewed elsewhere[124]. Given the role of activated fibroblasts in producing pro-inflammatory cytokines and chemokines, targeting of effector cytokines is being considered to prevent amplification of renal damage in fibrotic tissues[39]. For example, a role has been identified for IL-1 in promoting fibrosis via the induction of the transcription factor MYC in stromal mesenchymal cells[42], suggesting that IL-1 inhibitors could be tested for therapeutic efficacy in LN in the future. In addition, drugs that block

Table 2 | **Therapies currently in clinical trials for lupus nephritis**

Drug or therapy name	Targets	Trial phase	Drug mechanism	Results of previous studies	Refs
Mesenchymal stem cell therapy	Immune cell modulation	II	Multiple immune-modulating effects reported	Efficacy has been demonstrated in animal models and in uncontrolled studies in humans	151–154
Tacrolimus (FK506) and MMF	Immune cell suppression	IV	Additive or synergistic effect of the combination of a calcineurin inhibitor (tacrolimus) and an IMP inhibitor (MMF)	Improved remission rate, but increased adverse events	115
Voclosporin	Calcineurin	II	A calcineurin inhibitor that is more potent and less toxic than other members of this class	Phase III studies are pending following the success of phase II trials showing the benefit of adding voclosporin to MMF for remission induction	117,118,155
CFZ533 (iscalimab)	CD40	II	Non-depleting non-agonist anti-CD40 antibodies that inhibit T cell-dependent B cell responses	Previous studies of agents targeting CD40L were terminated owing to adverse thrombotic events; safety of CFZ533 has been demonstrated in other inflammatory diseases and in transplant recipients	132,156
BI 655064	CD40	II		Safety has been demonstrated in healthy volunteers and in patients with RA	132,157,158
KZR-616	Immunoproteasome	I	A small-molecule selective inhibitor that halts pro-inflammatory cytokine production without affecting normal T cell-dependent responses	Safety and tolerability reported in a phase Ib dose-escalation trial in patients with SLE	159,160
Obinutuzumab	CD20	II	A human anti-CD20 antibody that produces a more robust B cell depletion than rituximab	Phase II study showed improved remission rate when added to standard-of-care therapy; has received FDA breakthrough therapy designation for progression to phase III trials	121,161,162
BMS-986165	TYK2	II	An inhibitor of JAK family member TYK2 that inhibits IL-12, IL-23 and type I interferon signalling	Efficacy has been demonstrated in a phase II study of psoriasis	163,164
Anifrolumab	Type I interferon	II	An anti-IFNAR monoclonal antibody that blocks the binding of type I interferons to their receptor	Positive results reported in one of two phase III trials for general SLE	112,113,165
Belimumab	BAFF	III	B cell depletion and modulation	Has achieved primary and all major secondary end points in a phase III trial of LN	110,166
Eculizumab	Complement protein C5	NA	Anti-C5 antibody blocks the terminal complement pathway and C5 cleavage	Off-label use reported in refractory LN and thrombotic microangiopathy	36
Mizoribine	Nucleotide metabolism	III	IMP and GMP inhibitor	This drug is used extensively for LN in Japan but, as yet, there has not been a large-scale randomized controlled clinical trial	167,168
Iguratimod	NF-κB	II	NF-κB inhibitor	This drug is approved for treating RA in East Asia; testing in human LN is based on success in animal models and preliminary observations in patients with refractory LN	169–171
Secukinumab	IL-17	II	IL-17 inhibitor	Testing in LN is based on successful use of this class of drugs in other inflammatory diseases and the reported presence of T_H17 cells in the kidneys of patients with LN	172

BAFF, B cell-activating factor; CD40L, CD40 ligand; GMP, guanosine monophosphate; IFNAR, IFNα receptor; IMP, inosine monophosphate; JAK, Janus kinase; LN, lupus nephritis; MMF, mycophenolate mofetil; NA, not applicable; NF-κB, nuclear factor-κB; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T_H17, T helper 17; TYK2, tyrosine kinase 2.

PDGF receptors are currently being used to treat lung fibrosis and could also be considered for the treatment of fibrosis of the kidneys[125]. Finally, targeting of fibroblasts using chimeric antigen receptor (CAR) T cells has been applied in a mouse model of cardiac fibrosis and represents a new technology that could delay or reverse damage of fibrotic organs[126].

Importantly, patients with SLE have a high risk of premature cardiovascular disease[127], and CKD is an additional cause of endothelial dysfunction and increased cardiovascular risk[128]. A multi-targeted approach using lifestyle modification, angiotensin-converting enzyme inhibitors, statins and hypertension control can decrease the cardiovascular mortality associated with CKD and should be tested in patients with LN[129].

Conclusions

LN remains a challenging clinical problem. Some progress has been made in the past few years towards improving both risk assessment and monitoring in patients with LN, including improvements in genetic risk profiling, the identification of biomarkers for flare[73–75] and the development of patient stratification[60] and hazard index tools[71]. More work is required to address whether it is possible to detect preclinical LN and to design interventional studies that test strategies to prevent renal flares and/or CKD progression. In the USA, where non-white patient ethnicity is associated with poor outcomes for LN[3,20], there is an urgent need to understand the mechanisms underlying genetic risk of ESRD and to address socioeconomic disparities that might affect lifestyle choices and medication adherence.

The kidney is a highly complex organ with multiple cell types that interact with and support each other and that, with the exception of the tubular cells, has a limited capacity for regeneration. Interstitial inflammatory infiltrates are associated with both CKD and a poor prognosis of LN[45]. Our understanding of the various intrinsic and infiltrating cell types involved in renal injury is being advanced by single-cell analyses, with the goal of yielding new diagnostic and therapeutic strategies to improve the outcome of LN. As data become available from such studies, new technologies will enable a spatial mapping of the involved cell types and a closer analysis of the cell–cell interactions that contribute to renal damage. Although current therapies are highly focused on immune-modulating interventions, new developments in the general approach to CKD might be translatable to LN and could help to delay or prevent the terminal phases of the disease that are associated with tubular senescence, vascular impairment and fibrosis.

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Interplay between genetics and epigenetics in osteoarthritis

Sarah J. Rice[1], *Frank Beier*[2,3], *David A. Young*[1] *and John Loughlin*[1] ✉

Abstract | Research into the molecular genetics of osteoarthritis (OA) has been substantially bolstered in the past few years by the implementation of powerful genome-wide scans that have revealed a large number of novel risk loci associated with the disease. This refreshing wave of discovery has occurred concurrently with epigenetic studies of joint tissues that have examined DNA methylation, histone modifications and regulatory RNAs. These epigenetic analyses have involved investigations of joint development, homeostasis and disease and have used both human samples and animal models. What has become apparent from a comparison of these two complementary approaches is that many OA genetic risk signals interact with, map to or correlate with epigenetic mediators. This discovery implies that epigenetic mechanisms, and their effect on gene expression, are a major conduit through which OA genetic risk polymorphisms exert their functional effects. This observation is particularly exciting as it provides mechanistic insight into OA susceptibility. Furthermore, this knowledge reveals avenues for attenuating the negative effect of risk-conferring alleles by exposing the epigenome as an exploitable target for therapeutic intervention in OA.

Linkage disequilibrium
The non-random association of two alleles within a population. Alleles at multiple variants that are in linkage disequilibrium will frequently be inherited together and comprise haplotypes. Large regions of linkage disequilibrium, known as 'LD blocks', can occur when there is a lack of haplotype diversity.

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Osteoarthritis (OA) is a multifactorial disease with a major genetic component. In the past few years, a particularly large number of OA risk loci have been reported[1–5]. These impressive studies have involved the application of genome-wide association studies (GWAS) of millions of DNA variants, principally single nucleotide polymorphisms (SNPs), in large population cohorts of typically hundreds of thousands of individuals. Such insights have placed OA firmly in the polygenic category of common diseases. The most fruitful recent example is a study of the complete UK Biobank cohort, involving an analysis of over 77,000 patients with OA and 378,000 individuals without reported OA, which discovered 52 novel OA association signals[4]. Currently, over 90 OA susceptibility loci have been reported for Europeans or individuals of European descent, the ethnic group that has been most extensively investigated[1–5].

In silico analyses of the disease-associated DNA variants identified in OA GWAS, and of the co-inherited variants in strong linkage disequilibrium, have highlighted instances of risk-conferring alleles altering the amino acid sequence of a protein. Examples of such missense changes include variants in genes coding for cartilage oligomeric matrix protein (COMP), chondroadherin-like protein (CHADL) and type XI collagen, which are structural proteins of the cartilage extracellular matrix (ECM)[1,3]. However, the overwhelming majority of OA risk variants are not predicted to change the amino acid sequence of a protein but are instead located in non-protein coding regions of the genome; for example, in introns, in untranslated regions (UTRs) of mRNAs, or between genes in the large intergenic spaces. As such, OA genetic susceptibility is principally assumed to involve changes in the regulation of gene expression rather than changes in protein sequence. OA is not unusual in this regard, with the vast majority of common, multifactorial diseases also having a genetic architecture constructed of DNA variants that modulate the expression of target genes[6].

The availability of excised tissue following arthroplasty has provided researchers with the opportunity to directly test for correlations between OA risk alleles and gene expression in disease-relevant cells, including chondrocytes[7]. These studies typically compare the relevant contribution of risk alleles and non-risk alleles to the overall amount of mRNA expressed by a gene of interest in a process known as allelic expression imbalance analysis. This approach has led to the identification of a number of functional links between genetic risk of OA and target gene expression, including at the genes *ALDH1A2*, *MGP* and *PLEC*, with risk alleles correlating with increases or decreases in gene expression[8–12]. This approach has highlighted that regulation of gene expression is an important aspect of OA susceptibility and has uncovered regulatory elements involved in the disease, along with their target genes.

Key points

- Genome-wide association studies have uncovered a large number of novel osteoarthritis (OA) genetic risk loci in the past decade.
- The vast majority of these risk loci map to non-coding regions of the genome and are predicted to increase disease risk by modulating the expression of target genes.
- Many of these risk loci map close to or correlate with epigenetic mediators.
- Epigenetic features and mediators therefore represent a mechanistic link between OA genetic risk factors and the onset or progression of disease.
- Emerging genomic technologies, including assay for transposase-accessible chromatin using sequencing (ATAC-seq), genome editing and single-cell analyses, are starting to facilitate the interpretation of these epigenetic effects in OA.
- Epigenetic features are amenable to modulation and, as such, are potential therapeutic targets.

Epigenetics is the mechanism by which the genome alters the expression of genes without changing the primary DNA sequence[13]. The three major mechanisms of epigenetic regulation include chemical modifications to DNA (for example, methylation changes to cytosine–guanine dinucleotides (CpGs)), post-translational modification of histones and regulation by non-coding regulatory RNAs. All three mechanisms are active in somatic cells, can be transmitted through cell division and enable the cell to respond to the environment by altering the repertoire of expressed genes (either transiently or more permanently). These epigenetic mechanisms regulate the accessibility of chromatin, the binding of transcription factors to the DNA and the 3D organization of the genome.

A large number of epigenetic studies in musculoskeletal diseases, including OA, have been undertaken. Because of the ability to perform high-throughput analysis at relatively modest cost using arrays and next-generation sequencing, these studies have primarily focused on the analysis of DNA methylation and regulatory RNAs, in particular microRNAs (miRNAs). Several excellent reviews have been published recently on this topic, identifying epigenetic marks that correlate with disease and comparing epigenetic profiles between different tissues of an OA joint and across skeletal sites[14,15]. In this Review, we provide an update on the current status of OA epigenetics, focusing mainly on the interplay between genetics and epigenetics and how the latter informs us about the functional and mechanistic effect of OA genetic susceptibility.

Epigenetic regulation in joint tissues

When considering the role of epigenetic mechanisms in OA, two principle scenarios have to be distinguished. First, the development of articulating joints and the skeleton is regulated through a multitude of epigenetic mechanisms. Alterations in these processes can decrease or increase the risk of OA later in life, for example, by changing the joint shape (and the resulting biomechanical loading patterns), the cartilage ECM composition, the chondrocyte phenotype and/or the responsiveness of joint cells to cytokines and growth factors[16]. This scenario provides an example of a concept known as 'developmental origins of health and disease', where processes in prenatal and early postnatal development determine the baseline risk of disease[16]. Second, epigenetic processes can be triggered in adults by specific events, such as joint injuries or deregulated metabolism, and can control the gene expression changes that promote OA initiation and progression. To complicate matters further, some of the cellular changes associated with OA resemble those that are active during skeletal growth and development, such as a switch from an articular chondrocyte phenotype to a growth plate chondrocyte phenotype[16,17].

Distinguishing these various scenarios is challenging in samples from patients in late stages of disease; however, animal models enable us to examine the involvement of various components and integral pathways in development and disease independently. In this section, we discuss how epigenetic regulation affects both the development and pathogenesis of joint tissues, using data from patients and animal models.

DNA methylation. Methylation of DNA is associated with transcriptional regulation, most often involving repression of gene expression, and is the most widely studied epigenetic mechanism in OA. Strong evidence suggests that DNA methylation has a regulatory function in OA pathogenesis[18]. Analyses of individual genes as well as genome-wide approaches have revealed alterations in the methylation of many genes suspected to be involved in OA, including genes encoding transcription factors such as *SOX9*, genes encoding ECM proteins and matrix-degrading proteases including *COL2A1*, *ACAN* and *MMP13*, and genes involved in growth factor and cytokine signalling, such as *GDF5* and *BMP7* (REF.[18]). Although a systematic review of all the published studies has not been reported, some emerging trends suggest that methylation patterns can vary between different OA joints and between different stages of disease. For example, different methylation patterns can occur between cartilage of the knee and cartilage of the hip as well as between mildly and severely affected cartilage from the same joint[19–21]. Further integration of the disease-associated methylation changes in distinct joint tissues, including analysis of both RNA and protein expression data as well as histone modification patterns, is required to identify biologically meaningful changes and to obtain a deeper understanding of the mechanisms involved (BOX 1).

In addition to describing the methylation changes associated with joint development and disease progression, identification of the underlying mechanisms will be crucial in the future. In 2017, one study revealed an important function for the DNA methyltransferase DNMT3B in OA[22]. This enzyme is involved in de novo DNA methylation and is downregulated in both human OA and in a surgical mouse model of OA. Notably, cartilage-specific deletion of *Dnmt3b* in the mice resulted in early onset OA, probably mediated at least in part through alterations in cellular metabolism. By contrast, overexpression of *Dnmt3b* in cartilage protected the mice from surgically induced OA. Thus, DNMT3B has an important role in DNA methylation and is required for healthy maintenance of cartilage.

The removal of methyl groups from DNA can be critical for gene activation. Studies have shown that

Allelic expression imbalance
An imbalance in the relative amount of mRNA derived from each allele in a heterozygote individual, as measured by the use of a single-nucleotide polymorphism in the coding sequence or untranslated regions of a gene; any deviation from a 1:1 ratio (determined using DNA from the patient) implies that one allele is associated with a higher expression level than the other allele.

Box 1 | **Priority areas for future research in OA epigenetics**

- The inclusion of large sample sizes, both in terms of numbers of patients and numbers of epigenetic marks studied, in epigenetic studies
- The investigation of the 3D architecture of chromatin as a potential contributor to disease
- The study of additional joint tissues to cartilage, such as subchondral bone and the synovium
- Analyses of samples from young donors to investigate the potential developmental origin of osteoarthritis (OA)-associated genetic and epigenetic factors
- The integration of different genetic and epigenetic marks and correlation of these marks with other relevant measures such as protein expression data
- The continued investigation of the relationship between genotype, epigenotype, gene expression and phenotype
- The application of emerging technologies such as assay for transposase-accessible chromatin using sequencing (ATAC-seq), genome editing and single-cell analyses

patterns of hydroxymethylated cytosine (an intermediate of DNA demethylation) can change both in OA and during chondrocyte differentiation, indicating that DNA demethylation (and resulting gene activation) occurs during both processes[23,24]. Subsequent functional studies of chondrogenesis using cell lines and primary cells have implicated methylcytosine dioxygenase TET1 as an important enzyme in catalysing cytosine demethylation in chondrocytes[25].

Although large amounts of data regarding DNA methylation changes in OA are accumulating, we are only just beginning to identify some of the mechanisms of OA that involve these DNA methylation changes. Furthermore, whether these changes are causal or consequential in disease is not always apparent, and many areas require further investigation. For example, the majority of studies have focused on cartilage, with little attention paid to other relevant tissue types such as subchondral bone and the synovium. Studies that stratify by OA phenotypes are lacking, and genome-wide studies on cartilage and joint development are under-represented compared with studies on end-stage disease. Finally, more detailed mechanistic studies are required; for example, for defining the link between the different catabolic and anabolic regulators of DNA methylation that influence joint biology and for understanding the processes that govern the differential activation status of specific genes during OA.

Histone modifications. Post-translational modifications of histones through acetylation, methylation and other reversible chemical modulations regulate gene expression through a variety of mechanisms. These mechanisms include control of chromatin compaction, recruitment of transcription factors and cofactors, and provision of signals to readers of the chromatin code[26]. In contrast to genome-wide DNA methylation studies, most histone investigations have focused on examining the function of individual enzymes in cartilage development and/or OA. Chromatin immunoprecipitation sequencing (ChIP-seq) studies that characterize histone modifications on a genome-wide level are feasible, but have not yet been reported widely for OA. Chromatin status has been mapped for articular chondrocytes but dynamic changes during disease have not yet been assessed[27].

A balance between acetylation mediated by histone acetyltransferases (HATs) and deacetylation mediated by histone deacetylases (HDACs) regulates the acetylation status of histones. Notably, many of the HATs and HDACs also have additional substrates such as transcription factors[28]. Thus, the phenotypes observed upon knockout of genes encoding HDACs or HATs, or pharmacological modulation of the encoded proteins, cannot automatically be attributed to altered histone acetylation. Compared with HATs, much more information is available on the function of HDACs in joint biology. Histone deacetylation is generally associated with transcriptional repression and is catalysed by several classes of HDACs[29]. A number of class I and class II HDACs have been implicated in cartilage development; for example, knockout of HDAC3, HDAC4, HDAC5 or HDAC7 impairs endochondral ossification in mice[30–35]. Some of these HDACs have also been examined in OA in in vitro studies. For example, HDAC4 suppresses runt-related transcription factor 2 (RUNX2), a transcription factor important for chondrocyte hypertrophy, and MMP13 expression mediated by RUNX2 in chondrocytes from patients with OA[36]. However, the in vivo function of these proteins in models of OA is unclear and requires investigation. HDAC inhibitors have been tested as potential drugs to treat OA, on the basis that these compounds can inhibit the expression of genes encoding matrix degrading proteases and pro-inflammatory mediators[37,38]. However, caution is advised when investigating the potential applications of HDAC inhibitors, as non-specific HDAC inhibition could lead to activation of RUNX2 and chondrocyte hypertrophy.

Sirtuins (SIRTs) are a third class of deacetylases. In particular, the function of SIRT1 in the context of joint physiology has been extensively studied. SIRT1 is required for the maintenance of cartilage homeostasis[39–41], including through the regulation of mitochondrial biogenesis in chondrocytes[42]. Indeed, chondrocytes from patients with OA have a decreased mitochondrial biogenesis capacity, and activation of a pathway containing SIRT1 can reverse this phenotype in vitro[42]. Similarly, SIRT3-deficient mice develop spontaneous age-related OA associated with increased oxidative stress (indicative of mitochondrial dysfunction)[43]. In mice, loss of SIRT6 results in disturbed cartilage development[44] whereas overexpression of SIRT6 protects against surgery-induced OA[45]. In agreement with these data, SIRT6 depletion in human chondrocytes results in cellular senescence and increased expression of matrix-degrading proteases (MMP1 and MMP13)[46]. Collectively, these studies clearly demonstrate that histone deacetylation (and by extension histone acetylation) is essential in joint development, homeostasis and degeneration.

Following the first study to identify a genetic association between the histone methyltransferase gene *DOT1L* and OA[47], and subsequent functional studies of DOT1L in mouse models of OA[48,49], the role of histone methylation in OA pathology has attracted much attention. These investigations, which are discussed in more detail

Chromatin immunoprecipitation sequencing (ChIP-seq). A technique using antibodies and DNA sequencing to assess which proteins are binding to a DNA sequence, and/or which protein modifications are occurring, at particular points of the genome or genome wide; this technique can be performed on chromatin isolated from cell lines or cells from patients.

in a later section, have revealed a protective function for this enzyme in OA, including inhibition of chondrocyte hypertrophy. Similarly, deletion of the histone methyltransferase ESET in mice results in accelerated hypertrophic differentiation of both growth plate chondrocytes and articular chondrocytes, highlighting the overlap in mechanisms regulating the two cell types[50]. Mice lacking both EZH1 and EZH2, which are responsible for trimethylation of lysine 27 in histone H3 (H3K27; a repressive histone mark), have impaired growth plate development[51], and pharmacological inhibition of EZH2 slows progression of OA caused by transection of the anterior cruciate ligament in mice[52]. Inhibition of the histone demethylase KDM6B (also known as JMJD3) also impairs cartilage development and accelerates OA caused by destabilization of the medial meniscus in mice[53,54]. As discussed above for HATs and HDACs, one caveat of these findings is that histone methyltransferases and demethylases also have non-histone substrates, the modulation of which might be responsible for some of the described phenotypes.

Available evidence clearly supports the important roles of multiple histone-modifying proteins in joint development and homeostasis. However, much work remains to be done to identify joint-specific effects that could expose targets for therapeutic intervention. Comprehensive maps of multiple histone modifications during development and disease are required. Furthermore, and similar to the DNA methylation studies discussed above, most research has been carried out on cartilage, with little research yet performed on other tissues (BOX 1).

Non-coding RNAs. Non-coding RNAs, including miRNAs and long non-coding RNAs (lncRNAs), provide another level of epigenetic regulation of gene expression. The field of miRNAs in joint development and OA has grown considerably in the past decade, with >450 publications since 2009. miRNAs are being investigated as pathogenic contributors, therapeutic targets or agents, and potential biomarkers in OA. A comprehensive review of this topic is provided elsewhere[55–57] and is beyond the scope of this Review. In summary, strong evidence supports important functions of many miRNAs in joint development, ageing and disease, in both protective and catabolic roles, and in all joint tissues. Some of the important miRNAs in OA, such as miR-455 and miR-140, are discussed in a later section.

By contrast, much less work has been done on lncRNAs than on miRNAs, in particular in vivo, in part because of poor conservation of lncRNAs between species. In 2019, one study characterizing the expression of intergenic lncRNAs found that almost 200 of these lncRNAs are differentially expressed in the cartilage between healthy individuals and patients with OA[58]. A separate study focused on analysing synovial gene expression in patients with OA versus healthy individuals and identified 17 differentially expressed lncRNAs[59]. The functions of many of these RNAs are still unknown. However, injection of one lncRNA, HOTAIR, can induce features of OA in rats[60], whereas another lncRNA, LncRNA-HIT, is required for chondrogenesis during skeletal development in mice by promoting histone acetylation[61]. Furthermore, the lncRNA ROCR, which is expressed exclusively in cartilage, regulates chondrogenesis via controlling the expression of *SOX9* (as discussed in a later section); although ROCR is expressed in adult cartilage, and differentially expressed in OA, a functional role for this lncRNA in adult tissue remains to be defined[62]. Collectively, these data suggest that lncRNAs have important functions in both development and diseases of the joints, but this field is at a very early stage and much work needs to be done.

3D architecture of chromatin. Chromatin is not randomly distributed throughout the nucleus, but is instead packaged in a highly organized and dynamic fashion[63–65]. This highly regulated spatial organization controls features such as enhancer–promoter interactions, insulation of neighbouring genes from the effects of enhancers or silencers and co-localization of co-regulated genes within a subdomain of the nucleus. Chromatin organization changes during cell differentiation and in disease, but no study has examined such changes in OA or in cartilage development per se. However, a few studies have addressed chromatin organization during limb development, which has direct implications on the formation and function of joints. Loss of the transcriptional repressor CTCF, an important factor in controlling chromatin organization, in limb mesenchyme of mice leads to a complete absence of forelimbs and severe truncation of hindlimbs[66]. A series of landmark studies have shown that disruption of normal genome organization can lead to limb malformations in humans and in mice[67,68]. Although no evidence is yet available showing the effects of disrupted genome organization on OA, it is plausible that the 3D architecture of chromatin changes during ageing. This change might enable novel interactions between enhancers and promoters and alter gene expression patterns that promote development of the disease. Emerging evidence therefore suggests that 3D chromatin organization has an important function in the regulation of gene expression, tissue development and disease, and this process might contribute to the pathogenesis of OA (BOX 1).

Genetic–epigenetic interactions in OA

OA-associated SNPs and mQTLs. The identification of correlations between particular disease-associated SNPs and DNA CpG methylation patterns, known as methylation quantitative trait loci (mQTLs), is useful for the prioritization of effector genes and regulatory elements at genetic risk loci in common diseases[69]. These types of analysis can facilitate the interpretation of GWAS data, which frequently show the presence of complex gene-rich loci with no obvious link to disease pathology. mQTL analyses have been used in such a manner in a wide range of common traits, including OA, in which DNA from OA articular cartilage has been analysed[12,54,70–72].

To date, 18 genetic loci have been identified at which DNA CpG methylation patterns in cartilage correlate with genotype at OA-associated SNPs[12,70,71,73,74] (TABLE 1). One well-characterized example is an OA

Enhancers
Short sequences of DNA (<1,500 bp) that can 'activate' gene expression when bound by transcription factors by enhancing the activity of the gene promoter through physical interactions in *cis*.

Silencers
A sequence of DNA that can repress the expression of a gene through the direct binding of proteins that reduce or block transcription, which predominantly occurs through inhibiting the assembly of transcriptional machinery at a gene promoter.

Methylation quantitative trait loci
(mQTLs). Loci at which there is a correlation between the level of DNA methylation at a CpG site and the genotype at a single nucleotide polymorphism (SNP); mQTL assays are typically performed on DNA derived from cells from patients and can target specific CpGs and SNPs or can analyse the whole genome as part of a genome-wide approach, such as with CpG and genotyping arrays.

Table 1 | **mQTL analyses: OA-associated SNPs that correlate with CpG methylation**

OA-associated SNP	CpGs	Potential target gene	Function of encoded protein	Refs
rs6976	cg18099408 cg15147215 cg18591801	*GNL3*	Regulator of gene expression	70
rs10948172	cg13979708 cg19254793 cg20913747 cg18551225	*RUNX2*	Transcription factor involved in skeletogenesis	70,71
rs3204689	cg12031962	*ALDH1A2*	Enzyme involved in synthesis of retinoic acid	70
rs143383	cg14752227	*GDF5*	Chondrogenic growth factor	70
rs10471753	cg25008444	*PIK3R1*	Enzyme involved in synthesis of phosphatidylinositol	12
rs11780978	cg19405177	*PLEC*	Cytoskeletal linker protein required for tissue integrity	12
	cg20784950 cg01870834 cg07427475 cg02331830 cg04255391 cg14598846 cg23299254 cg10299941	*GRINA*	An ionotropic glutamate receptor	12
rs4764133	cg20917083	*MGP*	A calcium-binding protein secreted by chondrocytes to inhibit ectopic calcification	12
rs6516886	cg00065302 cg05468028 cg18001427 cg20220242 cg24751378 cg16140273	*RWDD2B*	Limited information currently available	12
rs11583641	cg18131582	*COLGALT2*	Enzyme that post-translationally modifies collagen	72
rs62182810	cg10114877	*NBEAL1*	Limited information currently available	72
rs11732213	cg20987369 cg25007799	*FGFR3*	Growth factor involved in skeletogenesis	72
rs9277552	cg02197634 cg25491704 cg13921245 cg02375585	*COL11A2*	Cartilage collagen	72
rs60890741	cg18170545	*ASAP1*	Regulator of cytoskeletal remodelling	72
rs317630	cg22375663	*CPSF1*	Component of the cleavage and polyadenylation specificity factor (CPSF) complex involved in processing pre-mRNAs	72
rs35206230	cg20040747 cg10253484	*SEMA7A*	Membrane glycoprotein involved in immune modulation	72
rs6499244	cg26736200 cg26661922	*NFAT5*	Nuclear factor of activated T cells 5 (NFAT5) is a transcription factor involved in the transcriptional regulation of osmoprotective and inflammatory genes.	72
		WWP2	WWP2 is an E3 ubiquitin-protein ligase involved in protein ubiquitylation. *WWP2* is also the host gene for miR-140, involved in regulation of gene expression	72
rs2953013	cg16779580	*RAB11FIP4*	A regulator of cell division	72

Table 1 (cont.) | **mQTL analyses: OA-associated SNPs that correlate with CpG methylation**

OA-associated SNP	CpGs	Potential target gene	Function of encoded protein	Refs
rs62063281	cg17117718	*LRRc37A*	Limited information currently available	72
	cg10826688	*CRHR1*	A receptor involved in signal transduction	72
	cg15295732	*MAPT*	Tau proteins involved in neuronal development	72
	cg11117266	*KANSL1*	A member of a histone acetyltransferase complex, involved in histone acetylation	72
	cg16520312			
	cg18228076			
	cg01934064			
	cg15633388			
	cg23616531			

CpG, cytosine–guanine dinucleotide; mQTL, methylation quantitative trait loci; OA, osteoarthritis; SNP, single nucleotide polymorphism.

risk locus located on chromosome 6p21.1 and marked by the SNP rs10948172 (FIG. 1). This SNP marks a topologically associating domain (TAD) containing only two genes: *SUPT3H*, encoding a HAT enzyme, and *RUNX2*, encoding a transcription factor essential for endochondral ossification and osteoblastic differentiation. Analysis of this risk locus in several different tissues from the articulating joint, including cartilage, identified a 572-bp differentially methylated region (DMR), 82 kb upstream of the two genes. At this DMR, the level of DNA methylation at CpGs correlated with the genotype at rs10948172, with the OA risk G allele being associated with lower levels of DNA methylation than the non-risk A allele[71] (FIG. 1). Furthermore, the expression of *RUNX2*, but not *SUPT3H*, correlated with methylation at CpGs within the DMR, marking out what is known as a methylation and expression quantitative trait locus (meQTL)[71]. Finally, CRISPR–Cas9 deletion of the DMR from the genome of Tc28a2 immortalized chondrocytes resulted in a fourfold increase in expression of the *RUNX2* P1 isoform, with no detectable effect on the expression of other measured transcripts[71]. This finding confirmed *RUNX2* as the target gene of the OA-associated mQTL at chromosome 6p21.1 (REF.[71]) and is of particular note in the context of OA aetiology, as *RUNX2* is known to be upregulated in OA cartilage and to contribute to chondrocyte hypertrophy, which is a common feature of the disease[73,74]. These types of OA mQTL analyses have resulted in the prioritization of many other genes with known or plausible functions in the context of OA aetiology including *PLEC*, *ALDH1A2*, *GDF5*, *MGP*, *COLGALT2* and *COL11A2*, through the identification of OA-associated regulatory elements, several of which have undergone downstream functional characterization[9,11,12,70,72,75]. In most instances, mQTL effects have been localized to regions predicted from chromatin state data to function as enhancers, placing OA in the 'enhanceropathy' category of common diseases[76].

One of the primary caveats of mQTL analyses carried out to date is the relatively sparse coverage of CpGs by arrays such as the Infinium HumanMethylation450 BeadChip array (Illumina, CA, USA) and its successor the MethylationEPIC BeadChip kit (Illumina), which cover approximately 1.6% and 3.0% of the 23 million CpGs in the human epigenome, respectively[77]. As a result, the report of 18 disease-associated mQTLs in OA (TABLE 1) is probably an underestimation of the true number. Future epigenetic analysis of genes and the corresponding regulatory elements at OA risk loci requires the generation of more comprehensive methylation data, including increased coverage of the epigenome, through sequencing-based technologies, which will provide a more accurate indication of the frequency with which DNA methylation functions as a conduit to differential gene expression in OA.

OA-associated SNPs and histone modifiers. A number of OA risk signals identified following GWAS have revealed loci that harbour genes encoding proteins that modify the human epigenome (TABLE 2; FIG. 2). This list includes genes encoding proteins that have a range of different functions relating to histone modification, including modification of the lysine methylation state (*PHF2* (REF.[78]), *PRDM9* (REF.[10]) and *DOT1L*[47,79]) or modification of the acetylation state (*SUPT3H*[4,10,47,79,80], *CITED2* (REF.[79]), *NCOA3* (REF.[78]) and *HMGN3* (REF.[78])) of the histone. Differential expression of any of these genes could result in an altered chromatin state, aberrant regulation of downstream genes and increased risk of disease. Furthermore, OA risk loci have been identified that harbour genes involved in the production of histones themselves, namely *HIST1H2AC*[4] and *SLBP*[4,79].

NCOA3 and *DOT1L* have been further characterized to establish their functional role in the context of OA pathology. Allelic expression imbalance of *NCOA3* was reported in the hip and knee articular cartilage of patients with OA who were heterozygous for the disease-associated SNP rs6094710, with the OA risk allele corresponding with a decrease in expression of the gene[81]. Immunohistological analysis revealed that NCOA3 is expressed within cartilage, and knockdown of *NCOA3* in human articular chondrocytes resulted in increased expression of the anabolic gene *COL2A1* and decreased expression of the catabolic genes *MMP13* and *RUNX2* (REF.[81]). *DOT1L* also has an integral chondroprotective function[48]. DOT1L is the only known eukaryotic H3K79 methyltransferase (an enzyme that mediates methylation of lysine 79 of histone H3)[82]. During cartilage homeostasis, the expression of DOT1L within the

Topologically associating domain
(TAD). Regions of the genome in which sequences of DNA can physically interact. Individual TADs are insulated by proteins such as CCCTC-binding factor (CTCF) and cohesin. These domains enable the regulation of target genes by their specific enhancers, while preventing the interaction of regulatory elements with genes outside the TAD.

Methylation and expression quantitative trait locus
(meQTL). A locus at which there is a correlation between the level of methylation at a CpG site and the expression of a gene, the latter being measured directly through quantitative reverse transcription PCR (qRT-PCR) or as part of a genome-wide approach, typically RNA sequencing.

Enhanceropathy
A pathology in which the underlying mechanism of disease involves aberrant function of gene enhancers. This pathology can be caused by altered chromatin state, DNA methylation or sequence variations within the enhancer region. Changes to the enhancer activity result in dysregulation of gene expression.

chondrocytes is associated with inhibition of SIRT1, coupled with H3K79 methylation at the promoters of the genes *LEF1* and *TCF1*, which mediate the nuclear response to Wnt signalling. Low-level transcription of these two genes enables low levels of active Wnt signalling to maintain cartilage homeostasis[48]. By contrast, a reduction or depletion of DOT1L within chondrocytes leads to high levels of Wnt signalling and cartilage degradation[48,49]. Notably, levels of H3K79 methylation are lower in chondrocytes from patients with OA than in chondrocytes from healthy individuals, and decreased activity of DOT1L in mice is associated with increased progression of both age-associated OA and post-traumatic OA[49]. Together, these studies provide strong evidence for the importance of the chromatin state of chondrocytes in the development and maintenance of healthy articular cartilage, and that aberrant regulation of chromatin modifications in OA can lead to cartilage degradation.

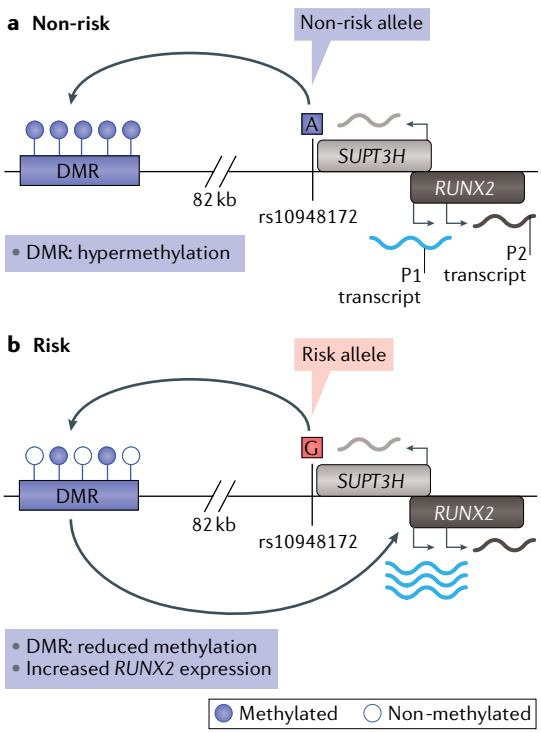

Fig. 1 | **Proposed mechanism of *RUNX2* regulation by an OA-associated mQTL.** The osteoarthritis (OA) risk locus marked by the single nucleotide polymorphism (SNP) rs10948172 contains two genes: *SUPT3H* and *RUNX2*. The gene *RUNX2*, encoding a transcription factor essential for healthy development and maintenance of the skeleton, has two distinct promoters: P1, producing the P1 transcript (blue); and P2, producing the P2 transcript (black). The P1 transcript is a longer mRNA molecule than the P2 transcript owing to the inclusion of an extra exon. **a** | At this SNP, the A allele (the non-risk allele) is associated with hypermethylation of DNA at distal cytosine–guanine dinucleotides (CpGs) (82 kb upstream of the genes) within a differentially methylated region (DMR). **b** | The G allele (the OA risk allele) correlates with lower levels of methylation within the DMR and is also associated with higher expression levels of the *RUNX2* P1 transcript, marking out an OA-associated methylation and expression quantitative trait locus (meQTL). This meQTL is notable as *RUNX2* is upregulated in the cartilage of patients with OA and hence is a good example of genetic and epigenetic interplay in OA. This figure is adapted from REF.[71], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

OA-associated SNPs and non-coding RNAs. A number of OA risk loci contain non-coding RNAs, including miRNAs located in introns of protein-coding genes (known as miRNA host genes). These risk loci include loci near *COL27A1* and *WWP2*, host genes for the cartilage-specific miRNAs, miR-455 and miR-140, respectively (TABLE 2). For both these miRNAs, the expression of the host gene and the corresponding miRNA are strongly correlated[83,84]. In 2019, one transcriptome-wide analysis identified the presence of allelic expression imbalance in *COL27A1* or *WWP2* in cartilage in OA[7]; however, the expression of the accompanying miRNAs was not analysed, and so whether these OA-associated SNPs also affect the expression of the miRNA is unknown.

For *COL27A1*, OA-associated SNPs are located within the promoter of the gene[4]. Mutations of *COL27A1*, which encodes a type of collagen expressed in the cartilage, are thought to underlie Steel syndrome, a rare Mendelian disorder of the skeleton that, among other phenotypes, results in short stature and hip pathology[85,86]. Similarly, mice with a large deletion in the coding sequence of *Col27a1* develop severe chondrodysplasia[87]. Notably, 6-month-old miR-455-null mice develop signs of early OA-like disease, with the presence of hypertrophic chondrocytes in thinner articular cartilage; however, a more detailed phenotypic analysis of these mice is needed[88]. Thus, again further studies are needed to determine whether the OA-associated SNPs within the promoter of *COL27A1* might function through influencing the expression of *COL27A1*, miR-455 or both.

A polymorphism at *WWP2* was associated with cartilage thickness[7] and with OA in a GWAS that combined data from the UK Biobank with an Icelandic cohort[3]. Intriguingly, in a second GWAS of the UK Biobank that combined data from the UK Biobank and from the Arthritis Research UK Osteoarthritis Genetics (arcOGEN) consortium[4], the same chromosomal region was identified as an OA susceptibility locus; however, the identified sentinel SNP (rs6499244) was located in the 3′ UTR of *NFAT5*, some 220 kb from the *WWP2* association signal, rs34195470 (REF.[3]). These two OA GWAS SNPs are in low linkage disequilibrium (pairwise r^2 0.22), implying that these signals are marking separate OA associations. Both genes have been implicated in arthritis, although *NFAT5* has only previously been linked with inflammatory arthritis[89]. Interestingly, rs34195470 is located within intron 10 of the full-length *WWP2*. This intron contains the promoter for both the C-terminal isoform of *WWP2* (*WWP2-C*) and miR-140 (REF.[90]), which might suggest that this *WWP2* transcript is processed to form miR-140. Intriguingly, although several transcript SNPs in *WWP2* showed allelic expression imbalance in cartilage, the strongest disease-associated signals came from SNPs within the WWP2-C/miR-140 transcript isoform[7].

Table 2 | **OA-associated SNPs in genomic regions encoding epigenomic regulators**

Association SNPs	Potential target gene	Function of encoded protein or RNA molecule	Refs
rs12551314	*PHF2*	Histone demethylase	78
rs1494593	*PRDM9*	Histone methyltransferase	10
rs12982744	*DOT1L*	Histone methyltransferase	47,79
rs11880992			
rs12154055	*SUPT3H*[a]	Histone acetyltransferase	4,10,47, 79,80
rs7770034			
rs10948155			
rs10948172			
rs7739938	*CITED2*	Co-activator of histone acetyltransferase	79
rs6094710	*NCOA3*	Histone acetyltransferase	78
rs1577792	*HMGN3*	Histone acetylation	78
rs115740542	*HIST1H2AC*	Histone	4
rs11732213	*SLBP*[a]	RNA binding protein that regulates transcription of histone mRNAs	4,79
rs2236995			
rs1078301	*COL27A1*[a] (host gene for miR-455)	Type XXVII collagen that functions as a structural element of cartilage (*COL27A1* also hosts the miRNA miR-455 involved in regulation of gene expression)	3,4
rs919642			
rs34195470	*WWP2*[a] (host gene for miR-140)	An E3 ubiquitin-protein ligase involved in protein ubiquitylation (*WWP2* also hosts the miRNA miR-140 involved in regulation of gene expression)	3,4
rs6499244[b]			
rs17659798	*MIR8068*	Intergenic miRNA involved in regulation of gene expression	4
rs11105466	*LINC02399*	Intergenic lncRNA involved in regulation of gene expression	4

lncRNA, long non-coding RNA; miRNA, microRNA; OA, osteoarthritis; SNP, single nucleotide polymorphism. [a]Independent association signals have been reported at *SUPT3H*, *SLBP*, *COL27A1* and *WWP2*, which might indicate that multiple risk loci operate on these genes. [b]This SNP is located in the 3′ untranslated region (UTR) of *NFAT5*.

In mice, deletion of *Wwp2* was associated with short stature and craniofacial truncation in an initial study[91], although subsequent studies of *Wwp2*-null mice did not replicate this skull phenotype[92–94]. miR-140-null mice are also short in stature and have a craniofacial truncation[95]; notably, a similar phenotype was reported in two unrelated patients with a heterozygous mutation in this miRNA[96]. miR-140-null mice and *Wwp2*-null mice also develop spontaneous OA-like disease, and the severity of the disease is increased in mice lacking both *Wwp2* and miR-140 (REFS[95,97]). Given the above findings, further work is clearly required to resolve whether the OA susceptibility loci within this region contribute to disease via regulating the expression of miR-140, *WWP2* or both.

The SNP rs8067763, which is associated with knee OA[4], is approximately 100 kb upstream of *SOX9*, which encodes a transcription factor important for skeletal development. During skeletogenesis, chondrocytes progress through a coordinated programme of maturation, controlled in part by SOX9, from resting chondrocytes, to proliferative chondrocytes, to pre-hypertrophic chondrocytes on to hypertrophic chondrocytes, and are eventually replaced by bone[98]. Temporal and tissue-specific expression of *SOX9* is regulated by a number of, often very distal, enhancers, chromosomal aberrations of which can cause the skeletal development disorder campomelic dysplasia[99]. Interestingly, although *SOX9* resides within a gene desert, rs8067763 is in close proximity to the cartilage-specific lncRNA ROCR. Deletion of *ROCR* prevents chondrogenic differentiation of mesenchymal stem cells (MSCs) in a mechanism that involves ablation of *SOX9* expression[62]. Whether ROCR regulates expression of genes in adult cartilage remains to be elucidated; however, SNPs in this region are also associated with hip shape and size[100], particularly of the intertrochanteric region[5], and thus are potentially associated with disease. As part of its function in cartilage, the transcription factor SOX9 also regulates the expression of miR-140 (REF.[84]), which itself mediates direct regulation (such as HDAC4 (REF.[101]) and ADAMTS5 (REF.[95])) and indirect regulation (such as MEF2C[102], RUNX2 (REF.[101]) and MMP13 (REF.[103])) of components involved in cartilage development and/or homeostasis (FIG. 3).

Some OA susceptibility loci are also located in close proximity to other potentially important non-coding RNAs, including miR-8068 and the lncRNA LINC02399 (REF.[4]). The function of these non-coding RNAs in the context of OA pathophysiology merits further investigation.

Emerging areas of exploration

Clear examples of functional and mechanistic interplay between OA genetic risk loci and epigenetics are now emerging. Some of these examples encompass a number of interacting genes and regulatory RNAs already discussed in the previous section of this Review. One excellent example is the interaction between *SOX9*,

Gene desert
A region of the genome that is devoid of protein-coding genes. These regions have been linked to several vital regulatory functions and might contain many spatiotemporal enhancers of important genes involved in development, such as *SOX9*.

Droplet digital PCR
A refinement of the conventional PCR method that uses a water–oil emulsion droplet system. Unlike traditional PCR, where a sample is amplified in a single reaction, droplet digital PCR has the benefit of increased precision through mass sample partitioning; the nucleic acid samples are partitioned into thousands of nanolitre-sized droplets, and PCR amplification is carried out within each droplet, ensuring reliable measurements of the DNA sequence being amplified.

ROCR, miR-140 and *RUNX2* (FIG. 3). To provide the ongoing capacity to detect these effects, and to discover new links between OA genetics and epigenetics, investigators will need to undertake larger and more comprehensive studies. For example, methylation analyses will need to include greater numbers of patients to provide the power to detect mQTLs in all risk alleles. The use of complementary genomic technologies will be necessary to maximize the interpretation of OA epigenetic datasets.

In this final section, we discuss four areas for novel investigation that are starting to be explored in the disease and that we think are particularly exciting.

Circulating regulatory RNAs and extracellular vesicles. Although the majority of genetic and epigenetic studies have focused on tissue-based expression profiling, which for OA is normally expression profiling in cartilage, interest is burgeoning in studying circulating RNAs, particularly miRNA, present in body fluids such as the serum and plasma. These circulating RNAs are present in circulating extracellular vesicles and exosomes, or in complexes with Argonaut proteins, the key effector proteins of miRNA-mediated silencing[104]. Such circulating RNAs are potential biomarkers of disease or disease status[105], but also have therapeutic potential. For example, pharmacological inhibition of miR-122 in non-human primates leads to long-lasting suppression of hepatitis C viraemia[106]. The purpose of such circulating RNAs is unclear, although they have been proposed to have a 'hormone-like' function in cell–cell communication[107]. A number of studies have thus examined the expression of circulating miRNAs in the serum or plasma of patients with OA; however, most of these studies had a number of limitations, including the use of samples from small numbers of patients[108–111], and no consistent miRNAs have been identified that predict either disease onset or progression. Nevertheless, even these limited studies suggest this area has potential, and further investigation is warranted with the use of miRNA profile screening in much larger and better-characterized cohorts, such as the Osteoarthritis Initiative (OAI). Quantitation and normalization of circulating miRNAs remains a challenge but methods based on droplet digital PCR could provide a solution for translation into clinical diagnostics[112].

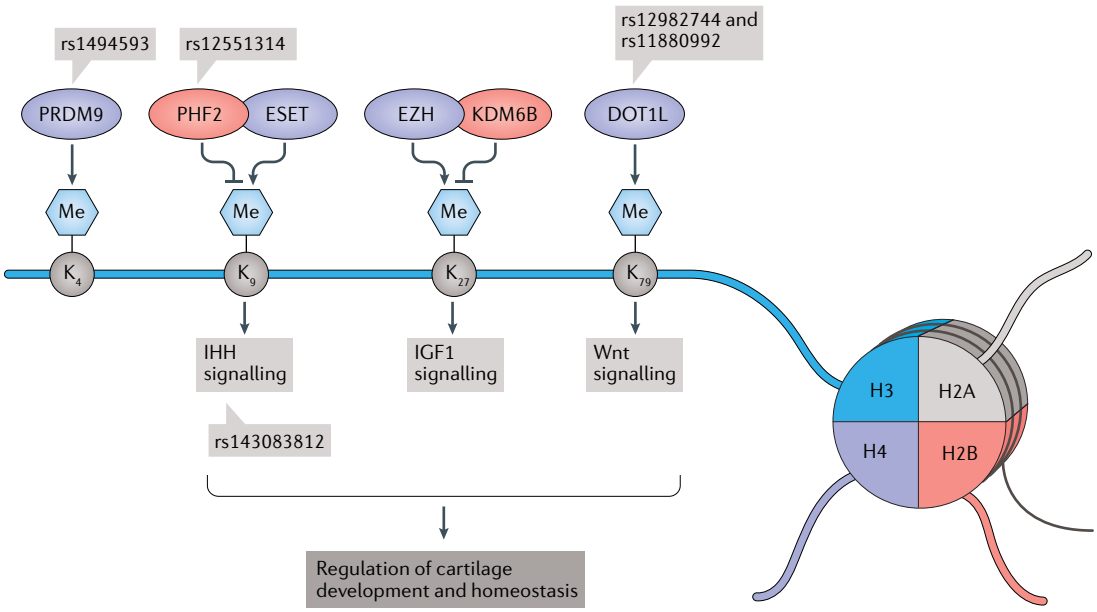

Fig. 2 | **Histone modifiers involved in cartilage development and homeostasis.** The basic repeat element of chromatin is the nucleosome that is composed primarily of histones. A nucleosome contains eight core histones: two each of histone H2A, H2B, H3 and H4. Each histone possesses an N-terminal tail that can undergo numerous post-translational amino acid modifications. The combination of these modifications constitutes a 'histone code' that is involved in the regulation of many aspects of cellular function. Specific lysine residues (K) can be modified by the enzymatic addition of acetyl (not shown) or methyl (Me) groups, the latter of which can involve the addition of monomethyl, dimethyl or trimethyl groups (not depicted in the figure for simplicity). These modifications can be enzymatically removed, in the case of a methyl group by demethylases. Many enzymes responsible for adding or removing these histone modifications are associated with osteoarthritis (OA) or cartilage development, genetically or functionally. For example, the methyltransferases PRDM9 (REF.[10]), ESET[50], EZH1 (REFS[51,52]), EZH2 (REFS[51,52]) and DOT1L[47,79] transfer methyl groups to lysine residues at the indicated position of histone H3, whereas the histone demethylases PHF2 (REF.[78]) and KDM6B[53,54] remove methyl groups from lysine residues. The consequences of histone modification are probably broad, but emerging evidence suggests that histone modifiers can affect a number of cellular pathways including Wnt signalling, insulin-like growth factor 1 signalling (IGF1) and Indian hedgehog (IHH) signalling, all of which are important for cartilage development and homeostasis, and all of which influence genetic susceptibility to OA[3,47–49]. These pathways are also directly influenced by genetic polymorphisms. rs numbers indicate GWAS-identified signals reported to affect the expression of these epigenetic modifiers or pathway components.

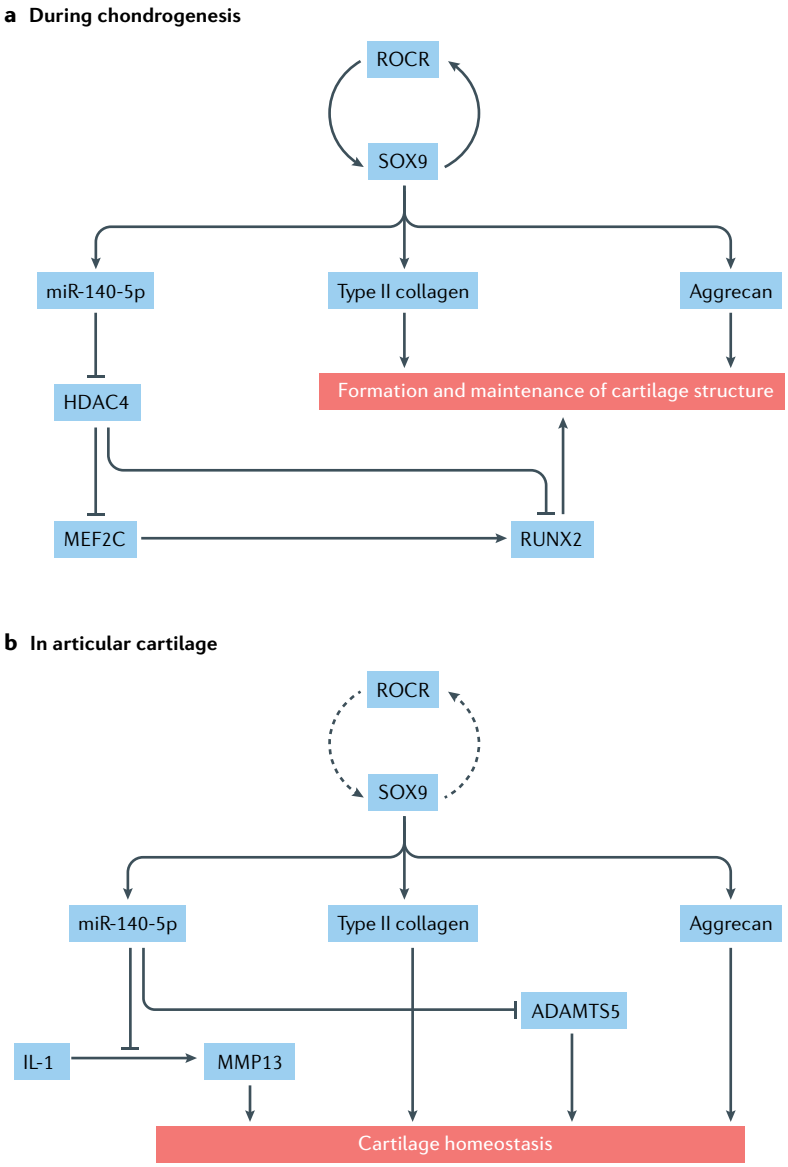

Fig. 3 | **Interactions between *SOX9*, ROCR, miR-140 and *RUNX2* during chondrogenesis and articular cartilage homeostasis. a** | During chondrogenesis, the long non-coding RNA (lncRNA) ROCR regulates the expression of *SOX9*, which reciprocally regulates the expression of ROCR. SOX9 is a key chondrogenic transcription factor that promotes the expression of many matrix genes including *COL2A1* and *ACAN*, which encode type II collagen and aggrecan, respectively, and are the major structural proteins of cartilage. SOX9 also promotes the expression of a microRNA (miRNA), miR-140, that indirectly affects the expression of *RUNX2* (encoding a transcription factor important for chondrocyte hypertrophy). miR-140-mediated regulation of *RUNX2* potentially occurs via inhibition of *HDAC4* expression, and thus this miRNA is involved in various aspects of endochondral bone formation. **b** | During cartilage homeostasis, a balance of anabolic responses (such as the production of type II collagen and aggrecan) and catabolic responses (such as the breakdown of cartilage structures by aggrecanases and collagenases, such as ADAMTS5 and MMP13, respectively) help maintain articular cartilage. Although ROCR is expressed in adult cartilage, and differentially expressed in osteoarthritis (OA), a functional role for this lncRNA in adult tissue remains to be defined but this lncRNA is hypothesized to control the expression of SOX9 (dotted line), which in turn regulates miR-140 expression. The dominant strand of miR-140, miR-140-5p, inhibits the expression of ADAMTS5 and also inhibits IL-1-mediated upregulation of MMP13 expression. Loss of cartilage in OA is typically mediated by an imbalance in these processes, favouring cartilage breakdown. Notably, miR-140-5p is downregulated in cartilage in OA. Many of these data are implied from mouse studies and further investigation in human tissue and samples is warranted.

Extracellular vesicles can themselves be used as a therapeutic tool to deliver cargo to target cells and tissues. MSC-derived exosomes can have protective effects in various models of cellular injury, although the underlying mechanisms remain to be fully resolved. In a rat osteochondral defect model, delivery of human embryonic MSC-derived exosomes restored the cartilage and subchondral bone[113]. The cargo of exosomes can also be modified in an attempt to improve their therapeutic efficacy; for example, injection of exosomes isolated from human synovial MSCs and modified to overexpress miR-140-5p, the perceived dominant strand of miR-140, remarkably reduced disease severity in a rat model of OA[114]. Thus, cell-free vesicles have great potential as future biomarkers and as a therapeutic delivery strategy, but the latter in particular requires further research and standardization.

Chondrocyte chromatin landscape. Although DNA methylation studies have proved useful for identifying genes or genomic regions that are differentially methylated in cartilage during disease or health, in reality, gene expression rarely correlates with alterations in DNA methylation at promoter regions. In fact, the tissue and/or cell-type specificity of gene expression seems to be regulated by enhancers[115], with enrichment of differentially methylated CpGs being observed in these elements in cartilage during both health and disease[116]. Furthermore, and as mentioned above, many OA-associated mQTLs in cartilage are localized to areas predicted to function as enhancers. Our current knowledge of the enhancers that are active in articular chondrocytes is, however, still quite limited and as such, research in this area should be prioritized. Active enhancers are considered to be enriched for the chromatin modification H3K4 methylation in combination with H3K27 acetylation[117]. Using ChIP-seq, these combined modifications have been used to define enhancers involved in chondrogenesis of MSCs[118] and to define the epigenome, including active enhancers, of adult chondrocytes, although these findings were in the context of early skeletal development[27]. Enhancer activity is associated with open chromatin regions, enabling transcription factors to bind and thus regulate gene expression. Hence, researchers can use a somewhat simpler technique than ChIP-seq, assay for transposase-accessible chromatin using sequencing (ATAC-seq; BOX 2), to map these open chromatin regions genome-wide. For example, in one study ATAC-seq was used to compare intact and damaged cartilage from patients with OA to define differentially accessible regions of the genome[119]. These regions were enriched in potential enhancers, differentially methylated loci and, of particular relevance, several OA-associated SNPs identified by GWAS.

Finally, although progress is being made in defining enhancers involved in the development and homeostasis of skeletal tissue, defining which gene or genes these enhancers regulate is problematic and many such analyses rely on investigating associations with the nearest genes. However, because of the previously described complex 3D architecture of chromatin, an enhancer can be far from its target gene and might interact with the

Box 2 | **ATAC-seq**

Short stretches of the genome are accessible to transcription factors and are thus probably important in gene regulation. Assay for transposase-accessible chromatin using sequencing (ATAC-seq) is a newly developed genome-wide method for identifying areas of the chromatin that are accessible to transcription factors. Although other techniques are available for measuring chromatin accessibility, often in more detail than ATAC-seq, the relative simplicity of ATAC-seq, along with the small biological sample (generally only 50,000 cells) required, have quickly made this method a standard tool in epigenetics and genome regulation research[137]. To this end, ATAC-seq analysis has been used to investigate a number of chromatin-related signatures in cell differentiation and development studies[138], and in diseases including cancer[139] and now osteoarthritis (OA)[119]. In ATAC-seq, nuclei are isolated from cells and incubated briefly with a modified transposase enzyme. This transposase cleaves accessible DNA in open chromatin regions while at the same time adding a specific DNA oligonucleotide at the cleavage site, a process termed tagmentation[137]. The thousands of DNA oligonucleotide-tagged genomic regions are amplified via PCR and sequenced. A range of bioinformatics tools are then used to map these regions, which, because of their relative enrichment, form peaks. Following the identification of these peaks, computational footprinting methods can be performed to identify enriched transcription factor binding sites that might promote cell specificity or disease activity. The simplicity of the ATAC-seq procedure has also made it the method of choice for examining the chromatin state at the single-cell level. In single-cell analyses, the use of ATAC-seq data to define enhancers results in better cell classification than the use of RNA expression data alone[140]. However, techniques to improve the mapping of chromatin modifications at the single-cell level are rapidly evolving[141].

gene via 'looping', bypassing intervening genes. A range of techniques, essentially variants based on chromosome confirmation capture (3C) techniques, can be used to determine the spatial proximity of regulatory elements to genes[120]. Although often imputed from well-studied model cell systems, such enhancer–gene interactions are spatiotemporally dependent and should thus be determined in the cell or tissue of interest. Such analysis is currently lacking in the OA field but has been applied successfully in the context of limb development[67,68]. Mapping the dynamic chromatin landscape earlier in disease, during development and in tissues other than cartilage is now warranted.

Modulation of epigenetic marks. The discovery of CRISPR sequences within the genome of archaea and bacteria, and the CRISPR-associated (Cas) proteins that comprise the prokaryotic adaptive immune system has been a revolutionary breakthrough in the field of molecular genetics. The CRISPR–Cas9 system now provides an indispensable tool for precise editing of the mammalian genome, and because of its cost-effectiveness, efficient targeting and ease of programming, this technique has become the gold standard in gene editing technology, largely replacing more established techniques such as zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALENs). Several published studies have used CRISPR–Cas9 in the study of chondrogenesis and chondrocyte pathobiology, principally through the direct knockdown of genes of interest or deletion of their regulatory elements[71,121–123].

Following the discovery of CRISPR–Cas9, the Cas9 protein has since been repurposed to produce a catalytically dead construct (dCas9) fused to a variety of effector elements. This approach has enabled targeted editing of the epigenome and direct activation or repression of gene expression (FIG. 4). One such modulator is the Krüppel-associated box (KRAB), which causes targeted trimethylation of H3K9, leading to transcriptional repression[124]. Targeting of dCas9–KRAB to the promoter regions of genes encoding receptors for pro-inflammatory cytokines, such as TNFR1 and IL-1R1, in human immortalized adipose-derived stem cells led to a reduction of up to 90% in expression of the genes, resulting in a lack of protein expression[125]. In the edited cells, this knockdown conferred protection against treatment with TNF or IL-1β, cytokines that can drive cartilage breakdown[125]. As these techniques continue to be optimized and developed, they should help elucidate the role of the epigenome in the maintenance of healthy chondrocytes and cartilage. In the short term, these tools will be useful for modelling disease to provide a better understanding of the underlying aetiology. Importantly, genome and epigenome modifications could be used to engineer 'enhanced' cartilage by altering OA-associated SNPs within the chondrocytes of an individual, and for targeted epigenome editing to optimize the epigenome, as the field moves towards personalized therapeutics and disease modification.

Single-cell analyses. Individual OA tissue transcriptomic datasets offer useful lists of differentially expressed genes and enriched pathways. The data portal SkeletalVis integrates these datasets and hence provides an exploration and comparison platform for skeletal transcriptomics data[126]. Although highly informative, these transcriptome datasets are the average expression signal of a bulk population of cells. By contrast, single-cell RNA sequencing (scRNA-seq) looks at individual cells and can identify novel cell populations, uncover regulatory relationships between genes and track the trajectories of distinct cell lineages in development and even in disease[127]. In terms of cartilage development, scRNA-seq aided in the identification of self-renewing and multipotent human skeletal stem cells that generate progenitors of bone, cartilage and stroma, but not fat[128]. These stem cells are present in adult bone and undergo fracture-induced expansion as a regenerative response to skeletal injury. The same stem cells can also be derived from adipose stroma, suggesting that adipose-derived stem cells could be used in the cell-based treatment of degenerative skeletal diseases. In terms of adult cartilage, the use of RNA-seq to analyse 1,464 chondrocytes from ten patients with OA enabled the identification of seven populations of chondrocytes in OA articular cartilage[129]. Furthermore, this analysis identified markers for distinguishing cartilage progenitor cells, cells with stem cell-like regenerative capacity[129,130]. Stratifying the patient samples on the basis of disease severity enabled the researchers to predict which genes contribute to OA progression and to define which chondrocyte population these genes are expressed in. With the current rapid improvement in scRNA-seq technology, future studies will probably involve much higher cell numbers as well as a combination of transcriptomic and epigenomic data, such as single-cell ATAC-seq analysis[127,131,132]. Although remarkable, single-cell analysis does involve disruption of the tissue, such as enzymatic digestion, to isolate individual cells. However, combining single-cell analysis

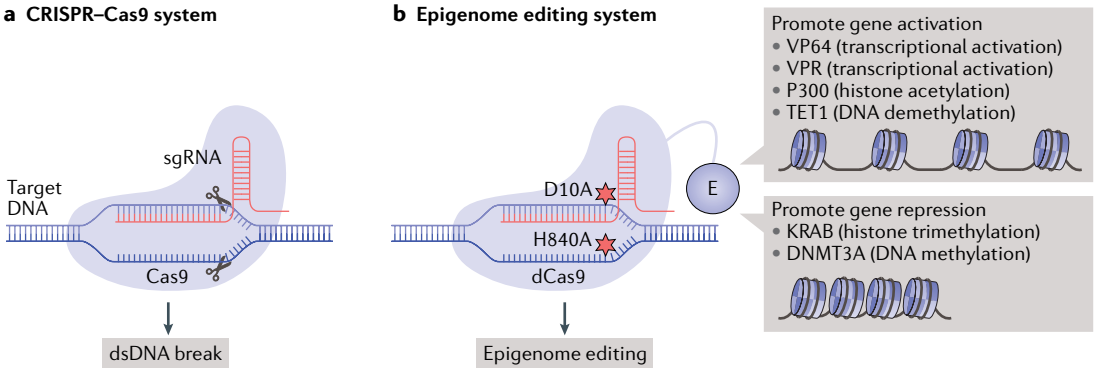

Fig. 4 | **The CRISPR–Cas9 system: repurposing for epigenome modulation. a** | The original CRISPR–Cas9 system is used by diverse species of bacteria and archaea and has been adopted as a gene-editing tool. The Cas9 protein is targeted to a specific region of the genome using a customized single guide RNA (sgRNA), and the two Cas9 nuclease domains (RuvC and HNH) initiate double-strand breaks in the DNA (dsDNA) at the target site. **b** | The catalytically dead (dCas9) system has two mutations in the nuclease domains (D10A in RuvC and H840A in HNH) to remove endonuclease activity of Cas9. The dCas9 protein functions as a DNA-binding domain that can be covalently bonded to effector proteins (E) to modulate the epigenome in the target region. Various effectors can promote either activation or repression of the target gene. DNMT3A, DNA methyltransferase 3a; KRAB, Krüppel-associated box; P300, histone acetyltransferase p300; TET1, methylcytosine dioxygenase TET1; VP64, viral protein 64; VPR, a second-generation activation domain consisting of VP64, the transcription factor p65, and replication and transcription activator (RTA).

(or datasets) with spatial transcriptomics should provide a positional context for the gene expression changes that occur during pathology[133].

Conclusions

By contributing to the regulation of the expression of a diverse range of genes, epigenetics has an important role in regulating the formation and maintenance of the joints. Furthermore, OA is associated with alterations in many epigenetic markers in affected tissues. Emerging data suggest that a large proportion of OA genetic risk loci have an effect on, or at least involve, epigenetic regulators. Hence, it is becoming clear that interactions between genetic risk loci and epigenetic factors, and consequential changes in gene expression, affect the occurrence and progression of OA.

The ability of articular chondrocytes to maintain a healthy phenotype is disrupted in OA[134], and a common theme to emerge from many OA studies is that epigenetic mechanisms have an important role in inhibiting the disease-causing transition of chondrocytes from an articular to a hypertrophic phenotype. As outlined in this Review, OA also has developmental origins resulting from aberrant joint morphology and ECM composition[16,135]. Epigenetic mechanisms are active during development and throughout life. As such, we should consider the possibility that identified OA genetic–epigenetic correlations might be exerting effects during joint formation and continue to exert effects throughout adolescence and into adulthood. OA might be an age-related disease, but the genetic and epigenetic risk could be functionally active from the beginning of life. If so, such a scenario could provide considerably more time for therapeutic intervention.

Future research should therefore include an expansion in the scale (more samples) and depth (more measures) of epigenetic and complementary analyses in a broader range of tissues (BOX 1). Furthermore, these analyses should be carried out not only in the adult tissues but also in developmental tissues, such as fetal samples. In addition, these data require continuous correlation with OA genetic risk loci to elucidate which loci are operating via the epigenome and which are therefore potentially therapeutically targetable. Finally, the use of machine learning and artificial intelligence technologies to integrate genomic, epigenetic, metabolic, proteomic and imaging data will enable a much more comprehensive understanding of OA pathogenesis, as well as patient stratification and the design of personalized treatments for individual patients[136].

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Spatial transcriptomics
A technique developed to quantify RNAs in cells without the need to isolate the cells or to homogenize the tissue, enabling investigators to discern spatial differences in gene expression in complex and heterogeneous tissues.

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Pharmacomicrobiomics in inflammatory arthritis: gut microbiome as modulator of therapeutic response

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Abstract | In the past three decades, extraordinary advances have been made in the understanding of the pathogenesis of, and treatment options for, inflammatory arthritides, including rheumatoid arthritis and spondyloarthritis. The use of methotrexate and subsequently biologic therapies (such as TNF inhibitors, among others) and oral small molecules have substantially improved clinical outcomes for many patients with inflammatory arthritis; for others, however, these agents do not substantially improve their symptoms. The emerging field of pharmacomicrobiomics, which investigates the effect of variations within the human gut microbiome on drugs, has already provided important insights into these therapeutics. Pharmacomicrobiomic studies have demonstrated that human gut microorganisms and their enzymatic products can affect the bioavailability, clinical efficacy and toxicity of a wide array of drugs through direct and indirect mechanisms. This discipline promises to facilitate the advent of microbiome-based precision medicine approaches in inflammatory arthritis, including strategies for predicting response to treatment and for modulating the microbiome to improve response to therapy or reduce drug toxicity.

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Inflammatory arthritides, including rheumatoid arthritis (RA), psoriatic arthritis (PsA) and ankylosing spondylitis (AS), are chronic, destructive, inflammatory disorders characterized by synovitis that can lead to accelerated morbidity, mortality and disability[1–4]. Over the past three decades, understanding of the immunological and molecular mechanisms in the pathogenesis of these disorders has advanced considerably, in particular with the discovery of TNF, IL-6 and other pro-inflammatory cytokines as important promoters of joint inflammation in RA, and of the role of TNF, IL-23 and IL-17 in spondyloarthritis (SpA; including PsA and AS) biology[5]. Furthermore, the use of methotrexate and, more recently, the advent of biologic therapies (such as those targeting TNF, IL-6 and the IL-23–IL-17 axis), as well as novel small molecules (for example, inhibitors of Janus kinase (JAK) or phosphodiesterase 4), has led to substantial improvements in clinical outcomes, ameliorating the quality of life for millions of patients with these forms of inflammatory arthritis. However, up to one half of patients with moderate or severe arthritis have no or suboptimal improvement in their symptoms with these treatments[6–13]. Therefore, insights into the underlying mechanisms that determine the pharmacokinetics and pharmacodynamics of anti-rheumatic drugs are urgently needed to maximize clinical response while eliminating patient frustration and wasteful health-care expenditure[14,15]. Multiple candidate biomarkers have been proposed for predicting (non)response to therapy, including clinical phenotype, host genetics, cytokines and autoantibodies, but they have either failed to be reproducible across cohorts or require lengthy treatment trials, during which joint damage could accrue.

Mounting evidence suggests that non-human genetic factors, most notably those derived from the trillions of microorganisms that live within and on the human body (the microbiota), might contribute to the development of RA and SpA in genetically susceptible individuals[16–18]. Although research examining intestinal communities as determinants of pathogenesis in inflammatory arthritis continues, the focus of study has also been expanded to include the mechanisms by which the aggregate genetic content of the gut microbiota (that is, the gut microbiome) encodes enzymatic machinery that modulates the pharmacokinetics of, and response to, immunomodulatory drugs[19]. The study of drug–microbiome interactions, termed pharmacomicrobiomics[20–22], builds upon extensive research dating back to the 1930s on how microorganisms affect drug metabolism[23,24]. Novel sequencing technology enables researchers to dissect

Key points

- Culture-independent, high-throughput DNA and RNA sequencing technologies —coupled with deeper insight into host mucosal immunology — have substantially advanced our understanding of the role of microorganisms in modulating health and disease.
- Pharmacomicrobiomics, an emerging field that describes the complex interaction of drugs with the microbiome, is increasingly considered an important factor in the prediction of therapeutic responses in many medical subspecialties.
- Multiple tools, including ex vivo cultures, metabolomics and gnotobiotic experiments, have enabled a deeper mechanistic understanding of host–microbial interactions in the pharmacokinetics of many available drugs.
- Emerging evidence supports the notion that the bioavailability, clinical efficacy and toxicity of several drugs used to treat human inflammatory arthritis can be modulated by human gut microorganisms and their enzymatic products.
- Pharmacomicrobiomics could potentially be incorporated into precision medicine approaches in rheumatology.

in ever more detail the constituent members of the gut microbiota and their genes and to investigate the effect of variations within the human gut microbiome on drugs; such research has already provided important insights into the effects of the microbiome on treatment response in autoimmunity and oncology, particularly those related to clinical outcomes of checkpoint inhibitors and biologic therapies[19,25].

In this Review, we describe evidence from studies in animal models and humans characterizing the dynamic interactions between the gut microbiota and xenobiotics, with special emphasis on pharmaceuticals relevant to rheumatology. We also discuss the tools available to study pharmacomicrobiomics and describe relevant translational data in cancer and autoimmune diseases, as well as ongoing work in RA and SpA. Last, we discuss strategies to incorporate pharmacomicrobiomics into the realm of precision medicine in rheumatology, with an emphasis on the development of tools to predict treatment response and the development of microbiome-derived adjuvant therapies.

Gut microorganisms in drug metabolism

From the earliest stages of life, humans ingest a multitude of xenobiotics, including a variety of chemicals and medications[26]. Immediately after birth, humans are rapidly colonized by trillions of microorganisms (collectively referred to as the microbiota), many of which will ultimately inhabit their gastrointestinal tract[27,28]. The microbiota has a variety of critical roles in human physiology: supplementing host nutrition, aiding metabolism (for example, by catabolizing dietary and host-derived polysaccharides into short-chain fatty acids)[29] and directly affecting maturation and development of the immune system and defence against pathogens[30,31].

Intriguingly, variability between individuals in the composition and metabolic competence of their microbiomes has a unique role in determining the clinical efficacy of (and development of adverse events associated with) some medications. This variability arises because specific, direct modifications of the chemical structures of ingested drugs are dependent on the composition of gut microbial communities and their collective enzymatic activity, which can differentially modify the bioavailability of these medications and ultimately determine their biological fate and clinical effects[32–34] (FIG. 1a). Within the umbrella concept of precision medicine, the study of drug–microbiome interactions (that is, pharmacomicrobiomics) is gaining traction. A long-term goal of this research discipline is to manipulate complex host-associated microbial communities to improve drug efficacy, predict treatment outcomes and reduce the development of adverse events. This concept is not foreign to rheumatologists, who were arguably among the first clinicians to apply pharmacomicrobiomics; as we describe, classic examples include the prodrug sulfasalazine (which requires cleaving by the gut microbiome in order to become an active drug)[35], as well as cyclophosphamide and methotrexate. Although fundamental insights into how gut microbiome-dependent biotransformations of xenobiotics affect human health are limited[36], numerous studies have highlighted the extent to which microbial xenobiotic metabolism varies between individuals, the mechanisms by which these microbial activities influence human biology and how these reactions can be logically manipulated for therapeutic purposes[37,38].

Notably, the collective gut microbiome-mediated modification of xenobiotics has a large metabolic component that is yet to be uncovered in its entirety[39]. The reasons for the vastness of this microbial enzymatic catalogue are multifactorial. The first reason relates to the greater abundance and diversity of bacterial cells relative to the more homogeneous host-intestinal cell population[40]. Equally important is the fact that gut bacteria are constantly subjected to evolutionary pressures exerted by the host and its ingested xenobiotics, which oblige the microorganisms to adapt to environmental fluctuations by altering their functional abilities and extracting vital nutrients for survival. These adaptations lead, in turn, to an extraordinary expansion of the number of xenobiotics that become subject to gut microbial metabolism[21,41].

These biotransformations occur through two main mechanisms (FIG. 1b,c). The first mechanism involves the direct interference of microbial enzymes with ingested medications, leading to the generation of end-products (or metabolites) that vary from the original prodrug. Several examples of this direct interference have been described[42], including the bacterial production of bioactive compounds (as in the hydrolysis of hydroxycinnamate esters by microbial cinnamoyl esterases)[43], microbial detoxification of drugs (for example, selected strains of the prevalent gut Actinobacterium *Eggerthella lenta* inactivate the cardiac drug digoxin)[44–47], direct interaction between microbial cells and xenobiotics (for example, physical attachment of *Helicobacter pylori* adhesins to levodopa, which decreases the bioavailability of the drug)[48] and interruption of the enterohepatic circulation and enteropathy of drugs (for example, inhibitors of luminal bacterial β-glucuronidase halt the hydrolysis of NSAID glucuronides and alleviate NSAID gut toxicity)[49]. The second mechanism involves indirect effects of host–microorganism interactions on drugs, including the alteration of host gene expression in response to microbial interactions[50], production of intermediate metabolites by gut microorganisms (for example, dietary-derived phosphatidylcholine conversion

Pharmacokinetics
The study of how an organism affects a drug, including absorption, distribution, bioavailability, metabolism and excretion.

Pharmacodynamics
The study of the biochemical, physiological and molecular effects of drugs on the body, including receptor binding, post-receptor effects and chemical interactions.

Xenobiotics
Chemical compounds (for example, drugs or pollutants) found within but not produced by living organisms.

Biotransformations
The processes by which a compound (for example, a drug) is transformed from one form to another by a chemical reaction within the body.

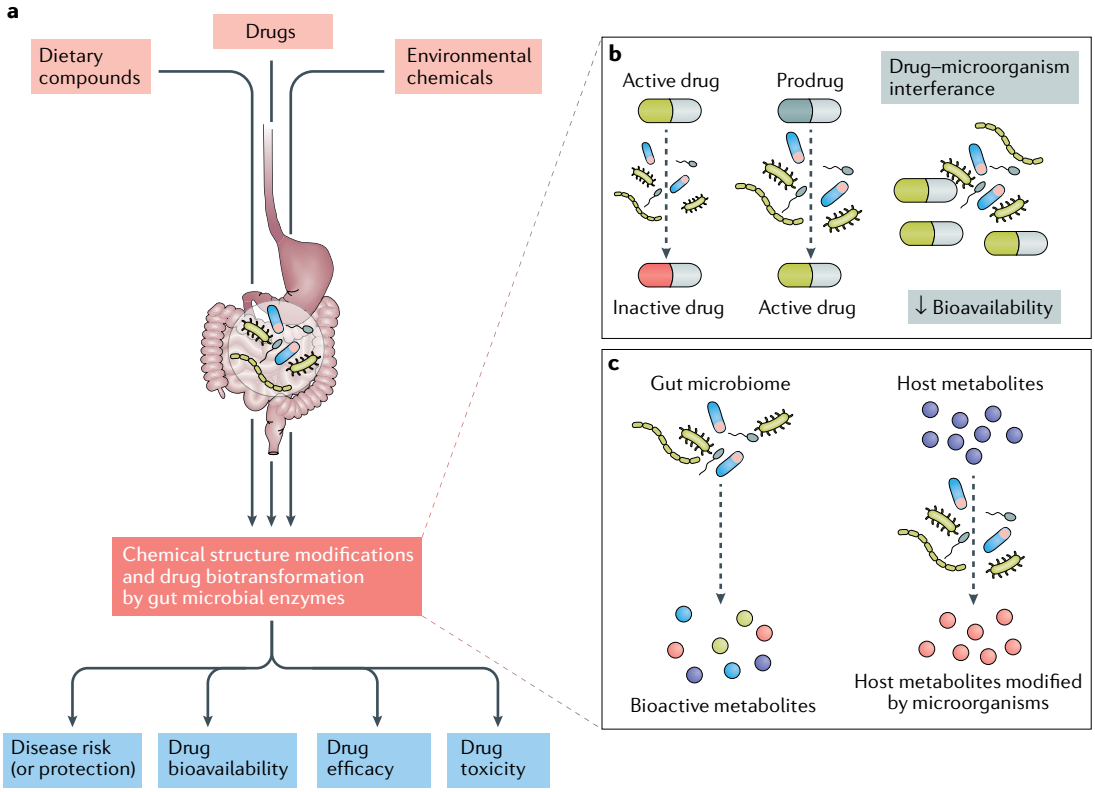

Fig. 1 | **Gut microorganisms in drug metabolism and physiology. a** | Bacteria and other microorganisms that inhabit the human gut can directly alter the chemical structures of many dietary components, environmental chemicals and pharmaceuticals. These biotransformations have the potential to affect drug bioavailability, pharmacokinetics, clinical efficacy and the development of adverse events. An accumulating body of evidence is clarifying the molecular mechanisms responsible for many of these biological changes in anti-inflammatory medications. **b** | Microorganisms can directly alter a drug through inactivation, activation or direct physical interactions that alter the drug's bioavailability. **c** | Indirect mechanisms of drug biotransformation include the production of intermediate bioactive metabolites by gut microorganisms and the alteration of host gene regulation and expression in response to microbial interactions.

by the intestinal bacteria to trimethylamine)[51–53] and competition between bacterial metabolites and drugs for binding sites in host enzymes (as in the case of bromovinyl uracil, a metabolite of the anti-viral drug sorivudine that inhibits the degradation of 5-fluorouracil, resulting in its accumulation in the blood and a marked increase in its toxicity)[54].

Tools to study pharmacomicrobiomics

Multiple methodologies are used to generate complementary lines of evidence implicating the microbiome in drug pharmacology. These approaches include the use of clinical studies, involving well-phenotyped cohorts with extensive clinical and demographic details, along with in vitro and ex vivo mechanistic experiments and studies in 'humanized' murine models[55] (BOX 1). This integrative approach has been critical in the identification of the microbial strains, microbial consortia, genes and/or metabolites necessary for drug biotransformation. The use of these methods was pioneered in original work exploring how gut microorganisms metabolize drugs such as digoxin and irinotecan[44,45,56,57].

A prototypical clinical study would involve samples obtained from a human population of interest (that is, individuals with a specific disease or clinical phenotype) and interrogate the microbiome at various taxonomic and functional levels, including the relative abundance of bacteria (using 16S rRNA gene sequencing (16S-seq)), gene families (metagenomic sequencing), microbial gene expression (metatranscriptomics) and metabolites (targeted and untargeted metabolomics). The results would then be analysed to characterize whether the pretreatment features (alone or in combination) of the microbiome correspond to a particular clinical outcome, most commonly in the form of efficacy or toxicity. Machine learning methods, including random forest and related decision-tree algorithms, could then be applied to create a predictive tool[25]. These analyses not only have the ability to rapidly inform clinical practice but also generate hypotheses regarding the mechanisms by which microbial transformations of drugs change their pharmacokinetic properties or lead to compound inactivation or prodrug activation.

Although patient cohort studies are critical for identifying associations between microbial factors and drug response, additional methods are required to provide causal evidence of microbially mediated drug metabolism. One such experimental approach employs the quantification of drug concentrations and related metabolites following the ex vivo incubation of the compound

Microbial consortia
Two or more microbial groups living symbiotically.

Random forest
A data construct classifier applied to machine learning that develops large numbers of random decision trees that analyse multiple sets of variables.

Box 1 | **Methods and tools for studying pharmacomicrobiomics**

Metagenomics
The study of a microbial community by sequencing the aggregate genetic material from an environmental or clinical sample. Abundance of drug-related pathways and/or specific enzymes within a microbial community can provide insights into non-host-driven biotransformation processes.

Gnotobiotic animals
Animals in which the composition of all microorganisms present is known; the term 'gnotobiotic' derives from the Greek words 'gnostos' (meaning 'known') and 'bios' ('life').

Germ-free mice
Mice bred and raised under conditions to render them free from all microorganisms. Transplanting whole human faecal microbial communities (or specific taxa or consortia) into germ-free mice enables the study of biotransformations within a known taxonomic environment.

Human microbiota-associated ('humanized') mice
Mice in which human faecal microbiota is established in germ-free mice through the transplantation of fresh or frozen gut microbiota samples (that is, faecal microbial transplantation).

Metabolomics
The quantification of all metabolites of a biological system, commonly using high-throughput analytical platforms such as nuclear magnetic resonance spectroscopy, gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry; in pharmacomicrobiomics, the focus is bacteria-derived and drug metabolites. Non-targeted metabolomics are optimized to cover as much of the metabolome as possible, whereas targeted metabolomics can accurately quantify a known set of metabolites.

Computational methods and machine learning
Integrative network analysis, pathway analysis and predictive models combine clinical phenotypic data, 16S rRNA gene sequencing data and metagenomic and metabolomic features to characterize interactions between drugs, the microbiome, metabolites and host factors and their effects on drug bioavailability and pharmacokinetics. These methods and models can then be used to predict clinical responses and the deleterious effects of medications of interest.

of interest with stool samples, microbial communities or specific bacterial strains under anaerobic conditions. Several platforms are commonly employed in drug metabolism and pharmacokinetics studies, including the many variations of mass spectrometry, most commonly liquid (or gas) chromatography–mass spectrometry and nuclear magnetic resonance spectroscopy. The application of these methods to analysing samples from patients enables the characterization of inter-individual variation in the rate of drug metabolism by gut microorganisms, comparisons between categories of clinical response or adverse events and hypothesis-generating research in model systems.

Whereas ex vivo profiling of human samples provides evidence for microorganism-mediated metabolism, in vitro studies are required to identify the bacterial genes or operons responsible for drug biotransformation. The recognition of which specific genes are involved in these biological processes requires the incubation of the drug of interest with bacterial strains, followed by comparative genomics and heterologous expression or deletion of key genes; such studies are capable of providing mechanistic insights into the role of the microbiome in drug metabolism[58].

A complementary in vivo strategy incorporates the use of gnotobiotic animals and humanized mouse models[59] (BOX 1) to further investigate the direct role of the microbiota in modulating drug pharmacokinetics. These techniques enable the study of intestinal microorganism–host interactions in human physiology, pathogenesis and pharmacology[60], while avoiding the confounding effects of commonplace variations such as host genotype and diet. In such studies, gnotobiotic mice are typically either germ-free animals or those colonized with defined microbiota[61], and humanization is achieved by transplanting whole human faecal microbial communities into germ-free mice, in order to interrogate biotransformations within a representative taxonomic environment. Experiments using germ-free animals are of course subject to a number of limitations, perhaps the most relevant of which is that the gut physiology of these animals is altered in comparison with wild-type animals, which in turn decreases their potential enzymatic and metabolic capabilities. However, humanization experiments can certainly help to explore the physiological effects of bacteria on the activation, inactivation and bioavailability of drugs in wild-type animals[19] or interrogate clinical outcomes in specific disorders[14,38] by the use of humanized murine (mouse and rat) models of autoimmune disease or inflammatory arthritis.

Drug biotransformation in mice

The pharmacomicrobiomics of several anti-rheumatic and immunosuppressive drugs have been studied in gnotobiotic experiments over the past few decades.

The prodrug sulfasalazine is considered the first rationally engineered medication for RA[62] and, curiously, it was developed synthetically to combine an antibiotic, sulfapyridine, with an anti-inflammatory 5-aminosalicylic acid (5-ASA) molecule[63] through an azo double bond. Sulfasalazine reaches the large intestine in its inactive form, where azoreductases encoded by the gut microbiome cleave the azo bond to release sulfapyridine and 5-ASA (FIG. 2a). Sulfapyridine is then almost completely absorbed to promote anti-arthritic effects, whereas nearly all of the 5-ASA is excreted and becomes the active compound for the treatment of ulcerative colitis[64]. The role of the intestinal microbiome in sulfasalazine metabolism was demonstrated in classic gnotobiotic studies in the 1970s, in which conventionally raised rats fully converted sulfasalazine into its constituent molecules, whereas antibiotics-treated or germ-free animals excreted mostly the prodrug[35]. Importantly, a consortium of four gut microbiota-derived bacterial strains was sufficient to re-establish sulfasalazine metabolism in these animals[35]. These findings were later confirmed by experiments utilizing ex vivo incubation of sulfasalazine with human faecal samples[65].

The fact that the murine gut microbiome alters methotrexate metabolism has been known for decades[66,67]; remarkably, the gut microbiome of mice can mediate the metabolism of methotrexate, producing glutamate and the inactive metabolite 2,4-diamino-N^{10}-methylpteroic acid (DAMPA) (FIG. 2b). Studies showed that, although methotrexate metabolites can be excreted and quantified in the faeces of conventionally reared animals, DAMPA is not detected in germ-free or antibiotics-treated mice, suggesting that the gut microbiome is necessary for this biotransformation.

Operons
Genetic regulatory systems found in bacteria and their viruses in which genes encoding functionally related proteins are clustered along the DNA.

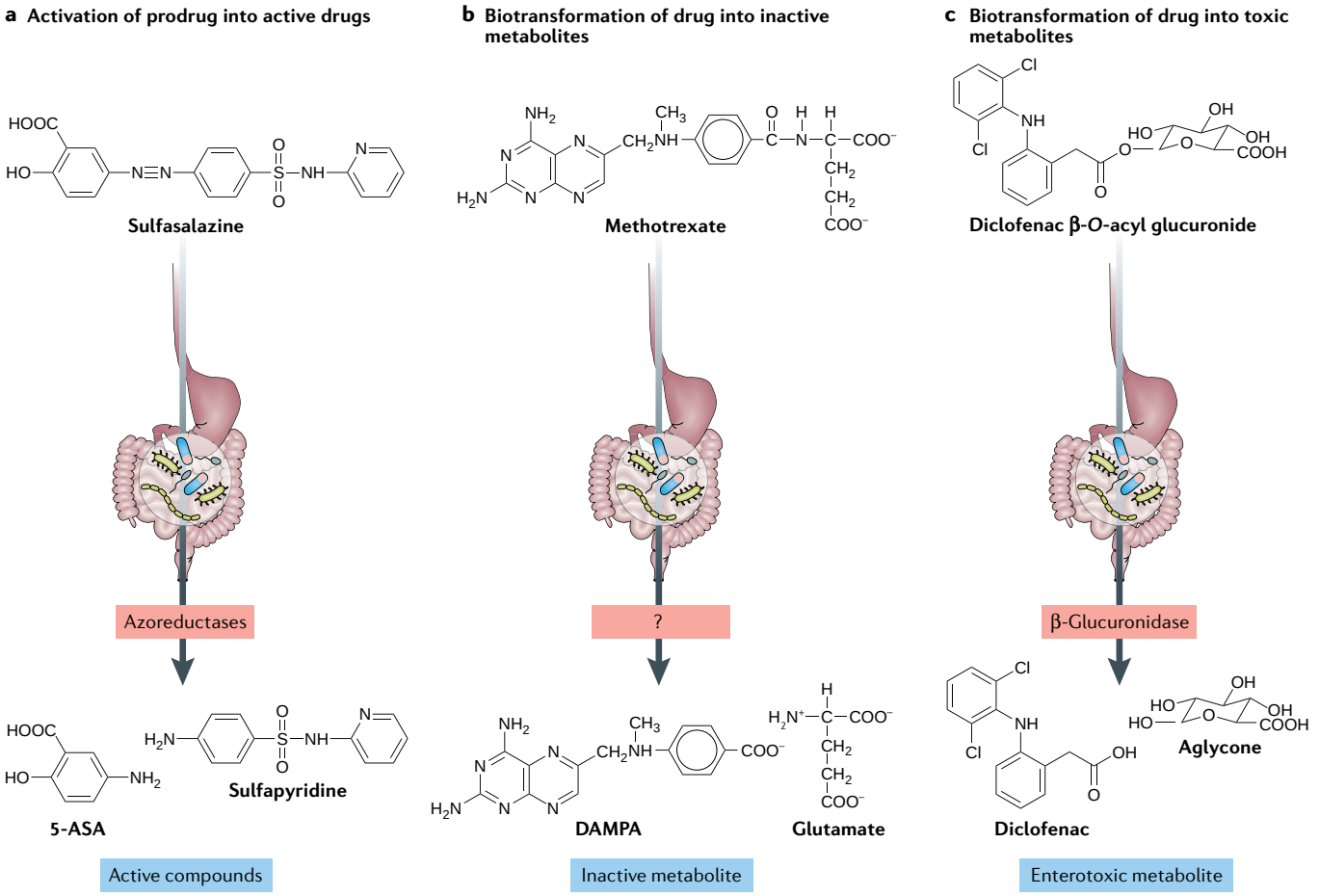

Fig. 2 | **Mechanisms of gut microbiome modulation of anti-rheumatic drug disposition and response.** The microbial metabolism of anti-rheumatic drugs can lead to their activation or inactivation, or result in the production of toxic compounds. **a** | Activation is the conversion of a prodrug into its bioactive form, thus contributing to therapeutic concentrations. For example, biotransformation of sulfasalazine produces 5-aminosalicylic acid (5-ASA) and sulfapyridine (the active form of the prodrug in rheumatoid arthritis). **b** | Inactivation is the conversion of an active metabolite into a less bioactive metabolite. For example, methotrexate is converted into 2,4-diamino-N^{10}-methylpteroic acid (DAMPA) through the action of an (as yet uncharacterized) microbial enzyme. **c** | Toxicity results from the production of bacterial metabolites that are deleterious to the host, for example, through the hydrolysis of glucuronidated NSAIDs.

The intestinal microbiome was also found to modulate the immunosuppressive effects of cyclophosphamide, a drug used for treating both arthritis and cancer[68]. Cyclophosphamide promotes a microbiota perturbation in the small intestine of cancer-bearing mice and induces the translocation of Gram-positive bacteria to secondary lymphoid organs, where they activate immune responses driven by pathogenic T helper 17 (T_H17) cells and memory T helper 1 (T_H1) cells[68]. However, under germ-free conditions (or after depletion of Gram-positive bacteria with antibiotics), these mice show decreased T_H17 responses and their cancer becomes resistant to cyclophosphamide, suggesting that the gut microbiota can help to shape the anti-cancer (and potentially anti-rheumatic) immune response to this drug and related compounds[69]. Although informative, these proof-of-principle, mechanistic studies were performed in mouse models, in which the microbiome composition differs substantially from that of humans. With this limitation in mind, subsequent work has looked at the generalizability of microbiome-mediated biotransformations in patients with rheumatic or oncological diseases.

Drug modulation by human gut microorganisms

Many of the initial studies in modern human pharmacomicrobiomics have been in immuno-oncology. These studies are of interest to the rheumatology field, as many drugs used in the treatment of cancer are either also used in rheumatology (for example, methotrexate and cyclophosphamide) or known to cause autoimmune-like syndromes (such as checkpoint inhibitor-induced inflammatory colitis or arthritis). Several examples elegantly illustrate how the gut microbiome can modulate response to therapy in human disease. A pivotal study in 2016 analysed outcomes in patients with metastatic melanoma undergoing treatment with the checkpoint inhibitor ipilimumab, a monoclonal antibody that blocks cytotoxic T lymphocyte antigen 4 (CTLA4), and correlated the pretreatment composition of the patients' microbiota with the development of colitis after treatment[70]. Baseline gut

microbiota composition also predicted colitis in a subsequent ipilimumab study[71], suggesting the possibility that microbial biomarkers might enable interventions to reduce the risk of inflammatory complications following immunotherapy.

Other work in the field of pharmacomicrobiomics has revealed that the baseline gut microbiome of patients with metastatic melanoma and other tumours can predict the outcomes of treatment with anti-programmed cell death protein 1 and anti-CTLA4 immunotherapies[25,72–74]. Importantly, modulation of the gut microbiome of germ-free mice via faecal microbiota transplantation (FMT) using samples from patients who responded to immunotherapy with ipilimumab could alter antitumour immunity and improve therapeutic response in the recipient mice[75]. Perhaps most intriguing is a 2018 report describing the successful implementation of FMT using samples from a single healthy unrelated donor to treat two patients with refractory immune checkpoint inhibitor-associated colitis; following FMT and gut microbiome reconstitution in both patients, the proportion of regulatory T cells increased within the colonic mucosa and clinical symptoms of colitis resolved[76].

A 2019 study[42] expanded our knowledge on the capacity of the human intestinal microbiome to biotransform oral medications prescribed for a wide range of clinical purposes, by combining the use of high-throughput functional genomic analyses and mass spectrometry to systematically identify human gut microorganisms and their gene products that metabolize drugs. Intriguingly, the results show that a large variety of human gut bacteria can metabolize a wide array of drugs, including anti-fungal, anti-hypertensive, anti-viral and hormone replacement medications; indeed, more than two thirds (176 of 271) of the tested medications were ultimately biotransformed[42]. However, the screening platform used in this study lacked controls, making the results and cut-off levels (that is, at what level a drug would be considered 'metabolized') challenging to interpret. Further work will be required to validate this approach.

Taken together, pharmacomicrobiomic data provide evidence that the gut microbiome can modulate the effects of parenteral immunotherapies and metabolize a sizable selection of oral medications (including anti-inflammatory drugs), with potential implications for the treatment of chronic inflammatory and autoimmune disorders[77,78].

Pharmacomicrobiomics in autoimmunity

Research groups investigating human autoimmune diseases have utilized pharmacomicrobiomics methods in the analysis of the intestinal microbiome and/or its genetically encoded functions as predictors of response to biologic therapies (TABLE 1). Three prospective studies using samples from patients with inflammatory bowel disease (IBD)[79] investigated associations between features of the microbiome and response to TNF inhibitors in biologic-naive patients with ulcerative colitis[80], the α4β7 integrin blocker vedolizumab in patients with IBD[81] and the IL-12–IL-23 blocker ustekinumab in patients with Crohn's disease[82]. Pharmacomicrobiomics has also been applied to the study of drugs used for the treatment of human rheumatic diseases[20,21]. For example, the metabolic fate of paracetamol (also known as acetaminophen) was shown to be markedly associated with an individual's pretreatment urinary concentration of p-cresol sulfate, a co-metabolite derived from the human gut microbiota[83,84]. As discussed, azo-bonded prodrugs used in the treatment of IBD and inflammatory arthritis

Table 1 | **Pharmacomicrobiomic studies in autoimmune and rheumatic diseases**

Disease	Study design	Intervention	Result	Ref.
Ulcerative colitis	Prospective	TNF inhibitors	Non-responders characterized by high dysbiosis indices and a lower abundance of *Faecalibacterium prausnitzii* at baseline	80
Ulcerative colitis and Crohn's disease	Prospective	Vedolizumab	High microbial diversity at baseline, specific taxa (e.g. *Roseburia inulinivorans*) and several microbial pathways enriched in patients achieving remission	81
Crohn's disease	Prospective	Ustekinumab	Patients achieving remission had high microbial diversity and enrichment of specific taxa at baseline	82
Axial SpA	Prospective	TNF inhibitors	High relative abundance of the order Burkholderiales at baseline was modestly predictive of future response	92
PsA and SpA	Prospective	TNF and IL-17A inhibitors	Abundance of several specific taxa (e.g. Clostridiales) shifted after treatment with IL-17 blockade (as compared with TNF inhibition); *Candida albicans* was expanded in a subset of patients following IL-17 blockade	94
Treatment-naive, chronic RA	Prospective	Herbal remedies with or without methotrexate	Oral microbiome (and to a lesser degree the gut microbiome) distinguished responders from non-responders	126
Treatment-naive, new-onset RA	Prospective	Methotrexate	Gut metagenome at baseline could differentiate methotrexate responders from non-responders; ex vivo incubation with methotrexate of samples from patients with treatment-naive, new-onset RA correlated with the magnitude of future clinical response	129

PsA, psoriatic arthritis; RA rheumatic arthritis; SpA, spondyloarthritis.

(including sulfasalazine) rely on colonic bacteria for cleavage of the azo bonds via microbial azoreductases, which releases the biologically active compound in the large intestine. These enzymes are ubiquitous across the human gut microbiome[85,86] and each azoreductase can bind multiple substrates[87,88]. However, the rate at which azo compounds are metabolized is substrate dependent. Moreover, the gut microbiota can metabolize the downstream metabolites of these azo reductions; for example, 5-ASA is inactivated by bacterial arylamine N-acetyltransferases[89]. Importantly, the activity of azoreductases has a high inter-individual variability[89,90], further underscoring the need to incorporate gut microbiome analysis and metabolomics when studying clinical disparities in drug efficacy. This need was exemplified in a study using an in vitro colonic simulator to determine the rates of metabolism of sulfasalazine and other azo-bonded prodrugs in the presence of human-derived colonic bacteria[65].

The intestinal microbiome has also been explored as a modulator of clinical outcome of treatment with monoclonal antibody therapies for inflammatory arthritis (TABLE 1). In 2018, a pilot study investigated whether baseline gut microbiota of patients with axial SpA predicted response to TNF inhibition[91]. Evaluation of stool samples from 19 patients using 16S-seq before and 3 months after anti-TNF treatment coupled with assessments of SpA disease activity suggested that a high relative abundance of the order Burkholderiales prior to initiation of anti-TNF therapy was modestly predictive of future response, although these results were not statistically significant after correction for multiple comparisons[92].

An intriguing study in the β-1,3-glucan (curdlan)-triggered SKG mouse model of SpA revealed that treatment of SKG mice with anti-IL-23 monoclonal antibodies before curdlan injection not only suppressed SpA development but also shifted the faecal microbiota composition (with an increase in the relative abundance of the families Clostridiales and Lactobacillaceae) and prevented the outgrowth of SpA-associated pathobionts[93]. These results suggest that the interplay between host IL-23 and gut bacteria might promote the emergence of clinically evident SpA in genetically predisposed individuals.

The gut microbiota is also perturbed in patients with new-onset PsA, with dysbiosis resembling that seen in patients with IBD[18]. Treatment with either IL-17 blockade or TNF blockade affects the gut bacterial and fungal microbiota of patients with PsA and SpA too[94]. The relative abundance of several specific bacterial taxa, particularly Clostridiales, shifted after both treatments, with the changes more prominent with IL-17 blockade compared with TNF blockade. Intriguingly, in a subgroup of patients, initiation of IL-17A blockade was associated with a perturbation of intestinal fungal taxa, most notably *Candida albicans*. These results are not unexpected, as most clinical trials have reported occurrences of oropharyngeal candidiasis after IL-17A blockade[95]. However, intestinal candidiasis could help to explain why this treatment strategy failed in IBD[96] and could potentially predict which (small subset of) patients with SpA treated with these biologics will develop (sub) clinical IBD[96,97].

Predicting response to methotrexate

Despite remarkable advances in understanding the pathogenesis of RA and the discovery of numerous new therapies, oral methotrexate remains the anchor drug for the treatment of RA and related autoimmune conditions worldwide[98]. An accumulating body of literature suggests that early and aggressive intervention with methotrexate results in low disease activity, slow radiographic progression and can even lead to remission in some patients with RA[99]. This principle is now reflected in current treatment guidelines for RA, most notably those from the ACR (published in 2015) and EULAR (2020), which recommend the use of methotrexate in all patients with early RA[100,101]. However, more than half of patients with moderate or severe RA show no or suboptimal improvement in their symptoms in response to methotrexate therapy[8,102–104], and bioavailability of the drug is known to be highly variable between individuals[105–107]. The reasons for these disparities remain unclear, and despite decades of study, differences in clinical response to methotrexate cannot be accurately predicted by host genetic factors or other established biomarkers[108]. An initial effort using concentrations of red blood cell methotrexate polyglutamates explained <20% of the variation in drug response[109,110] and required a lengthy trial of methotrexate treatment, but the findings have not been consistently reproduced in other cohorts[111,112]. Other factors explored as potential determinants of methotrexate efficacy have included serum or plasma concentrations of methotrexate[106,113], clinical factors such as sex and disease activity[114–117] and circulating $CD39^+$ regulatory T cells[117,118]. More than 70 genetic studies have also explored polymorphisms in candidate genes as predictors of methotrexate response, but no genetic marker has yet been sufficiently validated[119].

A handful of cohort studies have integrated clinical, demographic and host-genomic factors into models to predict (lack of) responsiveness to methotrexate[120,121]. More than a decade ago, pivotal work led to the first clinical–pharmacogenetic model (that is, combining risk alleles with sex, smoking and the presence of rheumatoid factor) to predict the efficacy of methotrexate monotherapy in patients with recent-onset RA (defined as disease duration <2 years)[122]. Although this tool has improved the original genetics-based models by integrating multiple variables, its accuracy remains imperfect, and the clinical application of this model is not generalizable across populations[123–125].

The failure of other factors to account for differences in the response to methotrexate raises the possibility that this variability could be driven, at least partially, by inter-individual disparities in the composition and function of the gut microbiome. As discussed earlier, work in germ-free and antibiotics-treated mice demonstrated decreased intestinal absorption and metabolism of methotrexate in these mice relative to wild-type mice[66,67], suggesting a critical role for the gut microbiome in the biotransformation of this drug. Moreover, the gut microbiomes of patients with untreated, new-onset RA have

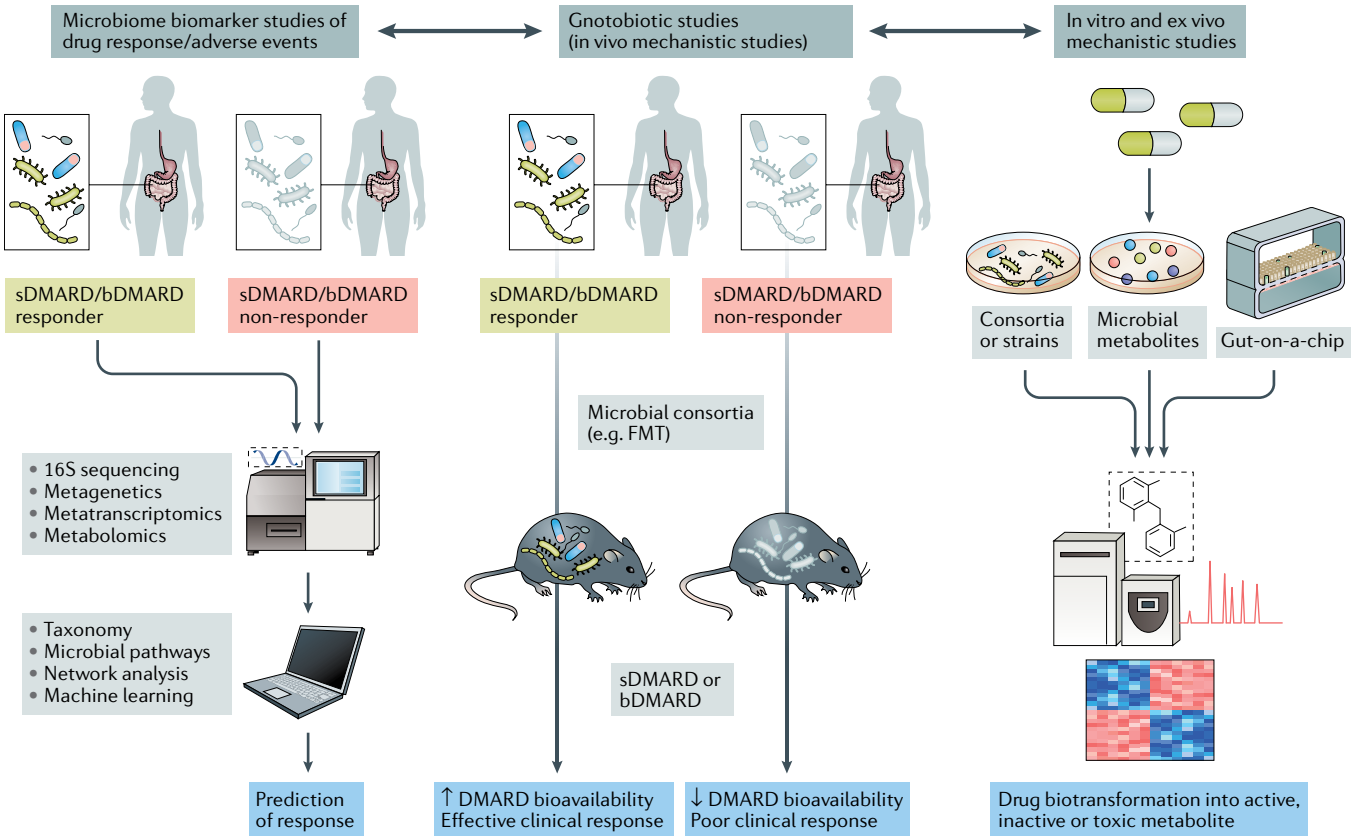

Fig. 3 | **Translational implications of pharmacomicrobiomic studies in rheumatic diseases.** In clinical studies with deeply phenotyped patient populations and known outcomes of synthetic (sDMARDs) or biologic DMARDs (bDMARDs) (such as efficacy and adverse events) (left panel), microbial features can be integrated with established biomarkers of response (for example, host genetics or immune cell profiles) via machine learning and network analyses to develop predictive tools. Mechanistic studies applying in vivo methods (middle panel) and in vitro or ex vivo methods (right panel) can complement and expand the understanding of drug biotransformation by the human gut microbiome, including activation, inactivation, conversion into toxic metabolites and bioavailability. FMT, faecal microbiota transplantation.

been found to vary in bacteria-derived purine metabolic pathways, including biosynthesis of tetrahydrofolate (and other purines)[17], which could modulate the absorption, bioavailability and downstream therapeutic effects of oral methotrexate.

A 2015 study found that the oral microbiome (and to a significantly lesser extent the gut microbiome) distinguished individuals with RA from healthy controls, and that microbiome alterations correlated with clinical indices and response to therapy, suggesting potential diagnostic and prognostic value[126]. However, this study focused primarily on patients with longstanding, established RA, who are known to harbour a markedly distinct gut microbiome relative to patients with new-onset RA[17]. In addition, response to methotrexate was predicted on the basis of the abundance of metagenomics-catalogued species rather than specific gene orthologues, thus precluding a detailed functional analysis.

A study using 16S-seq demonstrated that, over time, oral methotrexate at doses conventionally used in RA does not lead to consistent perturbations in gut microbial ecology[127]. However, applying in vitro and gnotobiotic methods, methotrexate can be observed to affect the composition of the gut microbiota of humanized mice in a dose-dependent manner and to directly inhibit the growth of some human gut bacteria[128]. Taken together, these data suggest that methotrexate, by altering bacterial physiology, might exert its anti-inflammatory effects in part by modulating the gut microbiome of patients with RA. Intriguingly, ongoing studies have demonstrated that the pretreatment microbiomes of patients with new-onset RA can be used to differentiate methotrexate responders from non-responders[129]. Moreover, use of machine learning techniques resulted in a robust predictive model, and remaining concentrations of methotrexate after ex vivo incubation with pretreatment samples from patients with new-onset RA correlated with the magnitude of future clinical response, suggesting a direct effect of the gut microbiome on methotrexate bioavailability and response to therapy[129]. Together, these results provide the first step towards the use of the gut microbiome to predict response to oral methotrexate therapy in patients with new onset RA and perhaps even its use as a target for manipulation in the treatment of rheumatic and autoimmune disease. Work is ongoing to understand if parenteral administration of methotrexate (and biologic therapies) can also be affected by the gut microbiome and whether using the microbiome as a predictor of response can be applied to other oral anti-rheumatic drugs (for example, JAK inhibitors).

Box 2 | **Potential applications of pharmacomicrobiomics in precision medicine**

Intestinal microbiome as a biomarker of response
Microbial community composition, the relative abundance of specific taxa, microbial pathways or metabolites could be measured to predict the efficacy and/or toxicity of synthetic and biologic DMARDs and other commonly used anti-rheumatic medications. This information could help to guide clinical decision-making and the initiation of early and effective treatments in rheumatoid arthritis, psoriatic arthritis and other related diseases.

Microbiome-modulating strategies
Taxonomic, metagenomic and metabolomic approaches enable the identification of microbial communities, strains and/or metabolites that can modulate drug bioavailability and improve clinical efficacy (or decrease the occurrence of adverse events). Strategies to modulate the microbiome include adjuvant therapies that either introduce communities or consortia (for example, via faecal microbiota transplantation or probiotics) or the modification of microbial composition through natural or engineered products (for example, probiotics).

Inhibition of gut microbial enzymes
Small molecules can be designed to inhibit the activity of bacterial functional pathways involved in the biotransformation of drugs into toxic metabolites (for example, the inhibition of β-glucuronidase to prevent NSAID-associated enteropathy).

Prebiotic
Non-digestible supplement that induces the growth (and/or activity) of commensal microorganisms.

Probiotic
Supplement containing live microorganisms that can alter the composition of microbiota and are supposed to provide health benefits to the host.

Bacterial culturomics
A method that allows for the description of the microbial composition by high-throughput culture platforms.

Applications for precision medicine

Advancing our knowledge and the translational applicability of pharmacomicrobiomics is highly relevant to our understanding of drug efficacy and adverse reactions to medications routinely prescribed in rheumatology (FIG. 3). Because the magnitude of response to drugs such as methotrexate, sulfasalazine and other synthetic and biologic DMARDs is known to have a high and unpredictable interindividual variability, the incorporation of precision medicine strategies based on features of the gut microbiome could help to guide a more rational use of these treatments (BOX 2).

From a diagnostic perspective, it is possible to envision the application of pharmacomicrobiomics in rheumatology through the measurement of microbial species, genes, transcripts and/or proteins that affect drug metabolism, small-molecule transport or immunoprotective responses[19]. This information could empower both clinicians and patients to adopt the best course of therapeutic action, on the basis of pretreatment gut microbial features (FIG. 3). In turn, this information can guide decision-making by either avoiding medications that are likely to fail to achieve meaningful clinical outcomes or engineer new avenues of microbiome-modulating strategies (sequential or adjuvant) that can lead to a desirable composition of microorganisms or genes to improve drug bioavailability and symptom amelioration. As discussed, these approaches have already proven successful in oncology (for example, the use of baseline gut microbiota as a predictor of clinical response and the development of colitis in checkpoint inhibitor trials[72–74], as well as the use of FMT for the treatment of colitis[70,71,130,131]), and they are now being employed in human inflammatory arthritis. One relevant example is the FLORA study[132], an ongoing randomized, placebo-controlled trial of FMT in patients with active PsA who have an inadequate response to methotrexate.

Although much will be learned from these proof-of-principle studies, other, less cumbersome, microbiome-regulating modalities are being tested, including adjuvant prebiotic and probiotic approaches that can potentially achieve similar results without the challenges and barriers of FMT (for example, risks inherent to the procedure, lack of clinical practicality and the potential to introduce pathogens into the recipient). Novel technologies, such as organs-on-chips (for example, gut-on-a-chip)[133–135] and bacterial culturomics[21,136], promise to aid in the understanding of the mechanisms underlying pharmacomicrobiomics by attempting to mimic the intestinal environment and to recapitulate physiological host–microorganism interactions. Drugs of interest can then be incubated in these systems to assess their effect on bacterial growth and metabolism[137], as well as the mechanisms by which bacteria biotransform medications (FIG. 3).

Conclusions

Numerous studies have demonstrated that the absorption, distribution, metabolism and excretion of drugs and other xenobiotics require multistep, effective interactions between host and microbial pathways[58]. Therefore, the integration of clinical factors, host genomics and pharmacomicrobiomics in a rigorous and validated manner, and their application in extensively phenotyped cohorts, will establish the basic knowledge for major advances in personalized medicine in rheumatology. Further discoveries of drug–microbiome–host interactions will require the application of innovative bioinformatic and machine learning tools coupled with ex vivo, in vitro and gnotobiotic models.

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Competing interests
J.U.S. declares that he has served as a consultant for Amgen, BMS, Janssen, Novartis, Sanofi and UCB, and has received funds from Novartis to NYU School of Medicine to conduct investigator-initiated studies. J.U.S. and S.B.A. have been granted USPTO patent no. 10011883 ("Causative agents and diagnostic methods relating to rheumatoid arthritis"). P.J.T. declares he is on the scientific advisory boards for Kaleido, Seres, SNIPRbiome, uBiome, and WholeBiome; there is no direct overlap between the current article and these consulting duties. R.R.N. and C.U. declare no competing interests.

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